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Transplantation of Kidney Carcinoma from Adult Frogs to Tadpoles*

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For practical reasons most tumor research is carried out on adult animals. These provide an environment for tumor growth which is expected to be different from that which would obtain in embryos and larvae. In the latter there is a progressive morphogenesis brought about by the action of morphogenetic "fields" or fields of induction (20). The limits of such fields and their mode of action have been particularly well worked out for amphibian development and regeneration. They appear to be absent from adult tailless amphibia and from most other adult vertebrates higher in the evolutionary scale. Aside from the presence of localized morphogenetic fields there must be, in the larval organism, some mechanism for correlating the relative growth of parts. In addition to these factors which are present in the embryo and larva, but are apparently absent from the adult (higher vertebrate), there are other points of difference. For example, in anuran amphibians the larva or tadpole differs from the adult in many respects such as habitat, diet, morphology, and endocrine function. It should be of interest, therefore, to study the behavior of a malignant tumor in an embryonic or larval environment possessing all of the mechanisms for controlling normal growth and morphogenesis.

The fundamental question to be approached by a study of this nature is the old one of whether the uncontrolled growth of the cancer cell is an intrinsic property of that cell, or whether the growth is due to the lack of suitable control mechanisms in the host. On the basis of animal transmission work and tissue culture studies it appears that the cancer cell maintains its structural and behavioral characteristics indefinitely and therefore must be an intrinsically altered cell. However, some cancers do not exceed certain normal tissues in growth rate and content of growth-promoting substances (2, 4, 17, 18). It is possible, therefore, that the alteration involved in the production of a cancer cell does not in itself produce rapid, uncontrolled growth. It could be, rather, that the change is just sufficient to release the cell from whatever controlling mechanisms there may be in the adult. If brought into an embryonic environment where growth controlling and morphogenetic mechanisms are known to be operating such a cell might be made to behave in a different and conceivably more normal fashion.

Suggestions of the same general nature as those outlined above have been made before by Waddington (19), Needham (16), and others. To my knowledge, however, little experimentation has been done directly on the problem. Needham (16) has reviewed the literature on the subject of tumors in embryos, larvae, and adult forms of lower vertebrates. More recently tumors have been induced in adult *Triton* (5) and *Rana pipiens* tadpoles (1). In most of these cases there is some doubt as to whether the tumors observed were truly malignant. One amphibian tumor, however, has been thoroughly studied and its malignancy conclusively demonstrated. This is the frog kidney adenocarcinoma of Lucké. In a series of papers (6-13) Lucké, and Lucké and Schlumberger have described this tumor and have shown that it metastasizes and is transmissible. The adult frog has lost the general capacity for organ regeneration, and for this and other reasons already mentioned provides an environment for the growth of the tumor which presumably is different from that which would obtain in the tadpole. With these points in mind we have transplanted the adult carcinoma to tadpoles to determine (a) whether or not the adult tumor will grow in the larva, and (b) what alterations, if any, are produced in the behavior and structure of the tumor when it is transplanted to various sites in the larva.

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MATERIALS AND METHODS

Rana pipiens adults were obtained from the northern Lake Champlain region in Vermont and lower Quebec. The animals were kept in a large slate aquarium tank in running tap water with the temperature at about 3° C. during the winter and increasing gradually to about 15° C. during May and early June.

The preparations for an experiment were made in the following way: A normal adult female was injected with one or two *Rana pipiens* pituitaries. After a day or two the eggs (now in the uterus) were stripped into a sperm suspension. After fertilization they were washed free of excess sperm and distributed to squat, half-gallon fish bowls (about 15 eggs to a bowl) where they developed into tadpoles. In most experiments they received the carcinoma implants after they had attained a total length of 18 to 22 mm.

Tumors were detected in donor frogs by palpation. Healthy parts of large tumors were washed three or fours times in sterile Ringer's solution and cut into small pieces about a millimeter in diameter. These pieces were placed in Ringer's solution in a covered dish which was kept cool in ice water. The tadpoles were then anesthetized in MS-222 [1] (0.016 per cent in Ringer's), placed in the operating dish on absorbent cotton in Ringer's, and the implantation performed under a binocular microscope. Each tadpole received a small piece of carcinoma tissue (0.3 to 0.5 mm. diameter) in one of the following sites: (a) subcutaneous tissue of the trunk just posterior to the ear, (b) mesenchyme of the dorsal tail fin, (c) adjacent to the kidney, (d) liver, (e) body cavity. Control implants of normal kidney tissue were made to the same sites. The details of the technic will be described in later sections of this report dealing with the results of implantation in each of these sites.

[1] MS-222 is a water-soluble anesthetic particularly useful for cold-blooded aquatic animals. It is obtainable from Sandoz Chemical Works, New York. For details, see E. ROTHLIN, Schweiz. med. Wchnschr., **62**:1042-1043. 1932.

After receiving the carcinoma or normal kidney control implants the tadpoles were placed in weaker MS-222 (0.005 per cent in Ringer's) for 4 to 6 hours at a temperature of about 10-15° C. They were then transferred to 50 per cent Ringer's solution but were left at 10-15° C. for one to two days until healing was complete. After this they were transferred to tap water at room temperature and feeding was resumed. The diet consisted of (a) a dried food made up of boiled liver, whole wheat flour, and milk; and (b) boiled lettuce. Following implantation the tadpoles were reared singly in finger bowls. Water was changed usually every second day.

The majority of the host tadpoles were allowed to develop through most of the larval period thus providing maximum time for growth of the implant. Tadpoles carrying tail implants were all autopsied before metamorphosis. Some of the tadpoles in other groups were allowed to metamorphose since we were interested in knowing if implants established during larval life would survive the period of transformation. All hosts were autopsied under the binocular microscope with careful examination of all organs, particularly the kidneys. The implant and the kidneys were fixed in "Susa" mixture, sectioned serially, and stained with Harris hematoxylin and either orange G or triosin.

TABLE I: THE INCIDENCE OF TUMORS DEVELOPING IN VARIOUS SITES IN TADPOLES FROM IMPLANTS OF ADULT FROG KIDNEY CARCINOMA

	Experimental hosts Tissue implanted = adult kidney carcinoma				Control hosts Tissue implanted = normal kidney		
Site	Total number of hosts	Tumors at implantation site	Tumors in kidney	Deaths	Total number of hosts	Tumors	Deaths
Subcutaneous tissue (trunk)	42	7	0	2	40	0	2
Dorsal tail fin	56	27	0	8	69	0	2
Adjacent to kidney	25	2	3	2	24	0	2
Liver	19	1	2	1	23	0	4
Body cavity	19	1	1	0	24	0	0
Totals	161	38	6	13	180	0	10

RESULTS

Table I summarizes the results of all of the experiments upon which this report is based. Kidney carcinoma tissue from 7 adult frogs was transplanted to 163 tadpole hosts. Two of the hosts died shortly after the operation and are not included in the tabulation. Thirteen tadpoles died during the course of the experiment. They were autopsied and are included in the following account of the results. One hundred and eighty-two control hosts received implants of normal kidney tissue from 7 adult frogs. Two of these hosts died after the operation and are not included in Table I. The 10 control hosts which died during the

course of the experiment are included in our account of the results.

At the time of autopsy careful examination was made of all of the fresh tissues under a dissecting microscope. Because of the characteristic form and color of the kidney adenocarcinoma it was possible to determine immediately whether or not tumors were present. The diagnosis made at this time was verified with the histological preparations. One hundred and thirty-seven sets of serial sections were studied. These included the implantation site from 39 experimental and 15 control hosts, and the kidneys from 51 experimental and 32 control hosts. In practically all cases a study of the sections verified the conclusions made from the examination of the fresh animal at autopsy.

Implants to the Subcutaneous Tissue behind the Ear

Just posterior to the ear in tadpoles there is a depression in the subcutaneous tissue located between the antero-dorsal portion of the celom and the postero-dorsal wall of the otic capsule. A small slit was made through the skin in this region and a pocket formed by pushing a small blunt glass rod through the slit into the subcutaneous tissue. The implant was then inserted in the slit with fine forceps and rammed into the subcutaneous pocket with the glass rod. Healing was complete within a day and all animals survived the operation. At the beginning of the experiment the tadpole hosts were 15 to 22 mm. in total length. They carried the implants for 3 to 5 months in most

Table II: Summary of Successful Subcutaneous Implants *

Host	Stage of development when autopsied	Elapsed time since implantation in days	Size of original implant: Mean diameter, mm.	Size of original implant: Volume, mm.³	Size of tumor at autopsy: Mean diameter, mm.	Size of tumor at autopsy: Volume, mm.³	Histological structure
3AKcE$_1$	35 mm. (tad)	196	0.4	0.034	8.5	320	Typical adenocarcinoma
3AKcE$_2$	Tr—21 (met) Ta—11	88	0.4	0.034	2.4	7.3	Adenocarcinoma †
4AKE$_4$	Tr—21 (met) Ta—31	136	0.6	0.11	2.0	4.2	Most of tumor regressing. Tubular ep. reduced, stroma increased. Contains a few typical growing tubules.
4AKE$_5$	22 mm. (frog)	129	0.5	0.066	2.4	7.3	Typical adenocarcinoma
4AKE$_{15}$	61 mm. (met)	130	0.3	0.014	1.0	0.5	Most of tumor typical adenocarcinoma. Some parts regressing.
4AKE$_{18}$	Tr—19 (met) Ta—3	129	0.5	0.066	1.3	1.2	As for 4AKE$_{15}$
4AKE$_{19}$	Tr—20 (met) Ta—12	172	0.4	0.034	2.4	7.3	Typical adenocarcinoma

* Site = subcutaneous tissue of trunk just posterior to right ear of tadpoles (15 to 22 mm. total length). The implants and the tumors developing from them were approximately spherical in shape. The mean of the three principal diameters was used in calculating tumor volumes according to the equation for the volume of a sphere. Abbreviations: Ta = tail, Tad = tadpole, Tr = trunk, Met = metamorphosing, Ep. = epithelium.

† Host dead at time of autopsy. Tumor has gross structure of adenocarcinoma but finer structural details were not preserved.

(A few minute tumor loci found in kidney sections had escaped detection at autopsy.)

The carcinoma implants produced tumors at the implantation site in 38 cases. In 6 cases there were no tumors at the original site but tumor loci were found in the kidneys. Unsuccessful carcinoma implants were gradually resorbed over a period of a few weeks. All of the implants of normal kidney tissue were similarly resorbed; both the implantation sites and the kidneys of control hosts were free of tumors.

Successful carcinoma implants produced tumors in all of the sites used. Some of these tumors grew to a large size (relative to size of host); all of them assumed the general appearance and manner of growth characteristic of adenocarcinoma in the adult kidney. In the following account the tumors occurring in each site are grouped together and described as a whole.

cases and were autopsied just before, during, and after metamorphosis.

Experimental series (42 cases).—The results are summarized in Table I. Seven out of a total of 42 animals developed tumors at the implantation site. The essential details for these positive cases are summarized in Table II. Six of the animals were autopsied during or immediately following metamorphosis. Each of these carried a tumor in the subcutaneous tissue. The tumor was posterior to the scapula in 3 cases, medial to it in 2 cases, and anterior to it in one case. The tumors located posterior or medial to the scapula caused an inwardly directed bulge in the anterior body wall. Thus they appeared to project into the body cavity but actually they did not penetrate the peritoneum and were entirely confined to the subcutaneous tissue.

All of the tumors were white or ivory-white in color, of firm consistency, and tended to assume spherical form except that the portion adjacent to the scapula was usually somewhat flattened. However, in making rough calculations of volumes (Table II) it was assumed that the growths were spherical. These calculations show a manyfold increase in the size of all implants during the course of the experiment. The histology of the growths differs somewhat in the various hosts. In two cases ($4AKE_5$, $4AKE_{19}$) the structure is that of typical active adult kidney adenocarcinoma. The main part of these tumors consists of the characteristic pseudostratified columnar epithelium in the forms of tubules and acini. The stroma is very sparse. There are no large cysts or regressing tubules. I should point out that one of these active tumors occurred in a tadpole undergoing metamorphosis while the other was found in a completely metamorphosed little frog. This is important in demonstrating that adult carcinoma transplanted to this site in tadpoles can maintain its structure during and after metamorphosis.

The tumors from animals $4AKE_{15}$ and $4AKE_{18}$ have the characteristic structure for the most part, but the stroma is thicker and some tubules are undergoing regressive changes. In parts of these tubules the columnar epithelium is reduced in height assuming cuboidal or even squamous form. Some cells are vacuolated; some contain pycnotic nuclei. These regressive changes are more evident in the tumor from animal $4AKE_4$. Here roughly half the tubules are completely regressed and the epithelium of most of the remaining tubules is flattened and contains some cells with large vacuoles and pycnotic nuclei. However, some of the tubules at the border of this growth are composed of the typical columnar epithelium showing mitotic figures. One tumor ($3AKcE_3$) was dead when fixed. It has the gross histology of adenocarcinoma, but finer details are lacking in the preparation.

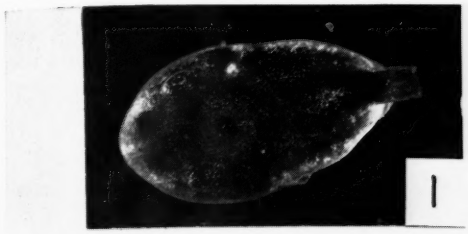
So far in this section we have considered only the growths found in animals autopsied during or slightly after metamorphosis. The one remaining case to be described demonstrates that subcutaneous implants of adult kidney carcinoma may grow to a large size in the tadpole. In this particular case ($3AKcE_1$) the implant was placed behind the right ear of a 15 mm. tadpole (Fig. 1). The host was considerably retarded in growth rate. Other animals of the same group metamorphosed in approximately 3 months while host $3AKcE_1$ was still a tadpole of 31 mm. total length. By this time the implant had grown considerably (Fig. 2). During the next 3 months the tumor continued to grow while the tadpole increased very little in size (Figs. 3-5). During the 7th month (196 days) the tadpole died but was fixed while still in a good state of preservation. The tumor was in the form of a sphere with the outer (lateral) surface somewhat flattened. Dimensions were 9.3 mm. from anterior to posterior, 9.1 mm. from dorsal to ventral, and 7.2 mm. from right to left. Taking the mean diameter as 8.5 mm. and assuming a spherical shape the volume is 320 $mm.^3$ Thus the volume of the original implant (0.034 $mm.^3$) had increased roughly 9,400 times. The tumor projected into the body cavity but was covered by peritoneum on its inner surface. The kidneys were serially sectioned but no growths were found. Other organs were also free of tumors. The histological appearance of this tumor is shown in Fig. 6. The major part consists of tumor tubules and acini proper. There are a moderate amount of supporting stroma and numerous cysts containing papillary extensions of the tumor epithelium.

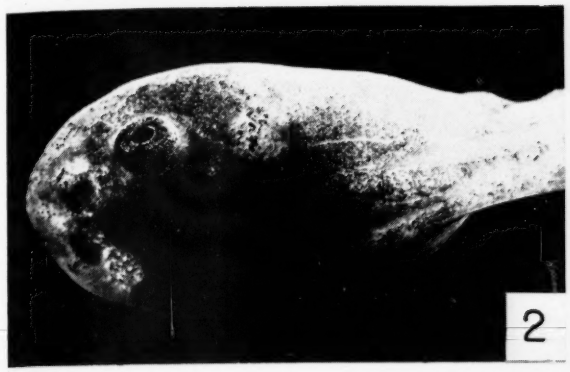
Control series (40 cases).—Conditions were exactly the same as those described for the experimental series except that the implants were of normal kidney tissue. All implants were resorbed; no tumors were found.

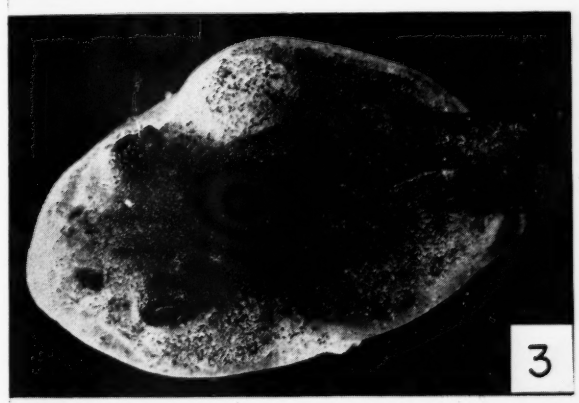
Implants to the Mesenchyme of the Dorsal Tail Fin

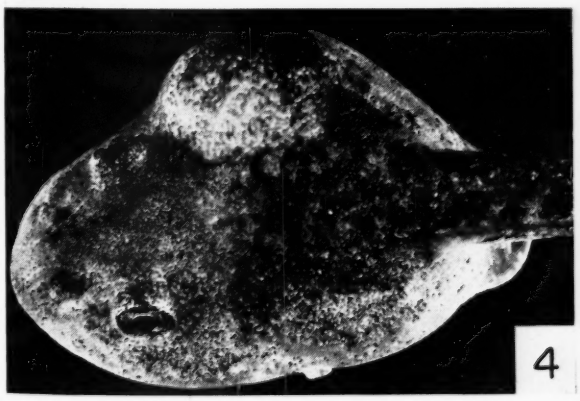
Tail implants were made according to the following technic: After the tadpole had been anesthetized it was placed on absorbent cotton in Ringer's solution with the tail lying flat on its left side and extending straight out from the body. The epidermis of the proximal third of the dorsal fin was pierced at one point on its right lateral surface with a small corneal knife and a pocket formed in the mesenchyme with a small glass rod. The implant was then placed in the opening with fine forceps and pushed into the pocket with the glass rod. Healing was usually complete within a day. Of a total of 125 animals which received implants only one died following the operation. At the beginning of the experiment the tadpoles were 19 to 21 mm. in total length except for 10 hosts (experiment 8) which were approximately 30 mm. long.

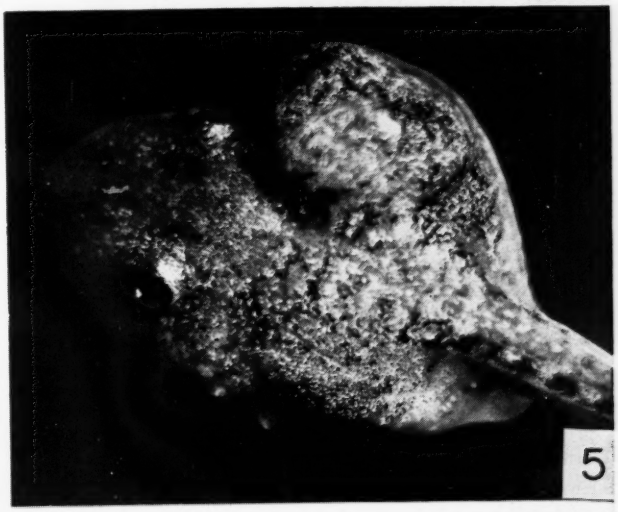
Experimental series (56 cases). Observations on living hosts.—Since the tail fin in most tadpoles is transparent, the fate of the implant could be followed in the living animals. Fifty-six tadpoles received implants of adult carcinoma tissue. In 4 cases the epidermis adjacent to the implant perforated within a few days after the operation and the carcinoma tissue was lost. The implant remained in place in 25 additional cases but failed to grow. In these tadpoles the carcinoma tissue rounded up into a sphere and the line of demarcation between implant and surrounding mesenchyme became indistinct. Over a period of several weeks the tumor tissue lost its dense whitish

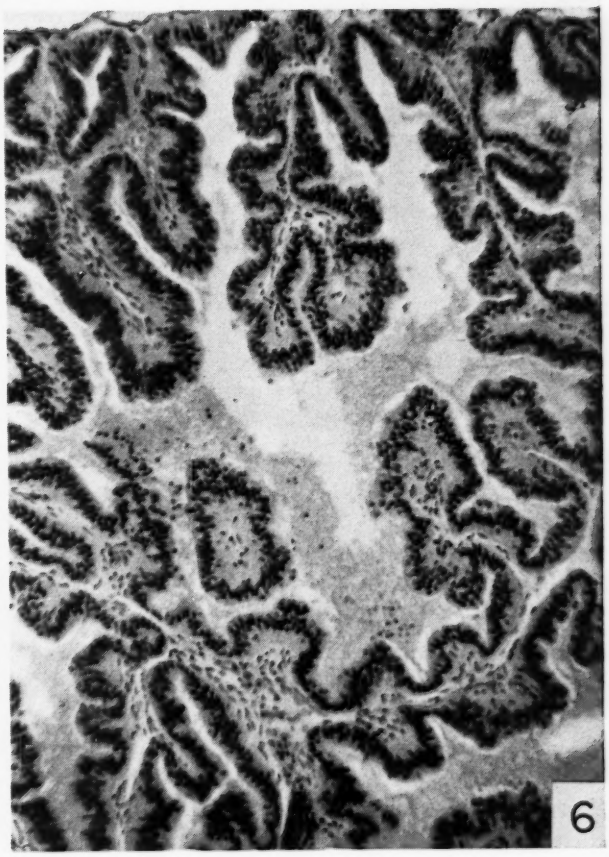












A series of photographs illustrating the progressive growth and the histology of subcutaneous carcinoma implants in tadpoles.

Fig. 1.—19 mm. host ($4AKE_1$) with 3-day implant. Mag. × 4.7.

Figs. 2-5.—Consecutive photographs of one host ($3AKcE_1$) showing the size of the implant at 100 days, 142 days, 170 days, and 183 days. Mag. × 4.7.

Fig. 6.—Section through the tumor shown in Fig. 5. Mag. × 115.

All photomicrographs were made by Mr. J. W. Pollock.

TABLE III: THE GROWTH OF ADULT FROG ADENOCARCINOMA IMPLANTS IN THE MESENCHYME OF THE DORSAL TAIL FIN OF TADPOLES *

Experiment	Total number of hosts	Number of takes	St., mm.	Mean diam. imp.	(A) Beginning of growth		(B) Maximum growth of implant			(C) Size of implant at autopsy		
					Time, days	St., mm.	Time, days	St., mm.	Mean diam., mm.	Time, days	St., mm.	Mean diam., mm.
4	15	9	19	0.4	39	38	95	51	1.6	133	61	1.3
				(0.4–0.5)	(20–55)	(35–43)	(58–123)	(41–61)	(0.9–3.3)	(104–138)	(57–61)	(0.0–3.3)
5	11	4	21	0.5	29	35	..	..	..	35	39	0.8‡
				(0.4–0.5)	(19–37)	(27–40)	..	..	..	(19–58)	(27–56)	(0.5–1.2)
6	5	2	20	0.5	20	38	33	46	0.9	68	57	0.6
				(0.4–0.5)	..	..	..	(45–48)	(0.8–1.0)	(64–72)	(53–60)	(0.5–0.7)
7	20	11	20	0.4	26	42†	33	44	1.0	81	57	0.4
				(0.3–0.5)	(22–26)	(38–45)	(26–42)	(38–51)	(0.7–1.3)	(62–103)	(50–62)	(0.0–1.1)
8	5	1	30	0.6	..	..	36	51	1.5	56	65	1.0

* The times and stages are given at which the implant (A) begins to grow, and (B) attains maximum size. By the time of autopsy (C) most of the implants had regressed in varying degrees (see text). The figures in parentheses represent the limits of variation in the data. Abbreviations: diam. = diameter, st. = stage of development in terms of total length in millimeters, imp. = implant. Note: Experiment 4 was run during the spring and early summer at room temperature (20-23° C.). Experiments 5 to 8 inclusive were run during the summer at higher temperatures (24-27° C.) and the tadpoles accordingly developed more rapidly than in experiment 4.

† Growth of implant already initiated.

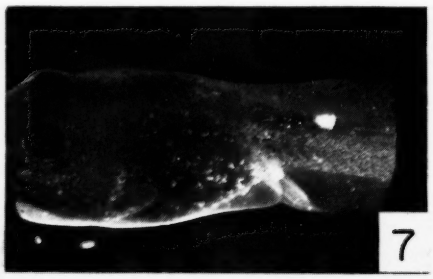
‡ Three of the four hosts in experiment 5 were autopsied shortly after beginning of growth of implant.

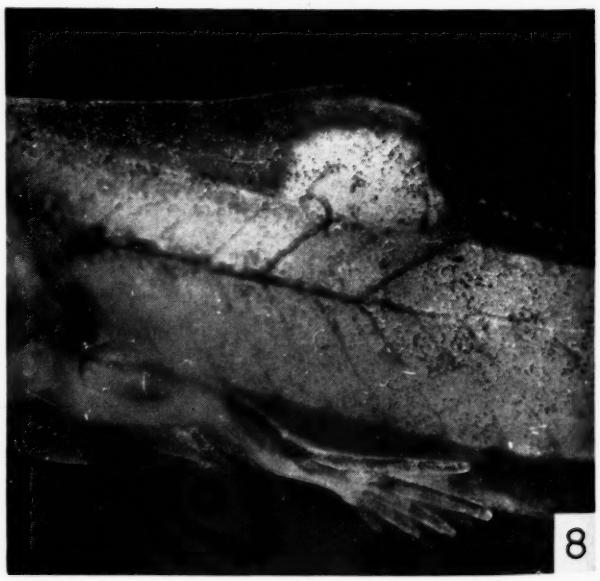
appearance, gradually became more transparent, and was finally replaced by rather dense mesenchyme containing a few melanophores.

In 27 cases there was significant growth of the implanted carcinoma tissue (Table III; Figs. 7, 8). During the first month following implantation the carcinoma tissue remained intact and sharply marked off from the surrounding mesenchyme, but showed no gross signs of growth. Camera lucida drawings during the 4th, 5th, and 6th weeks showed a definite increase in size. At this time most of the tadpoles were approximately 35 to 40 mm. in total length. The implant was well defined and of dense whitish appearance.

Practically all of the growth of the tail implants took place between the 35 to 40 mm. and the 45 to 50 mm. stages. In all hosts the growth followed very much the same pattern. The dimensions increased almost equally producing a tumor the shape of which approximated a sphere. Some implants were located at the base of the dorsal mesenchyme adjacent to the tail muscle. In these, growth ventrally was restricted and the tumor assumed a hemispherical form. The gross appearance was that of typical kidney carcinoma. The growths were compact, dense, whitish, and sharply marked off from surrounding tissues. The surface was rather shiny and often irregular due to uneven outward extension of tumor tubules. Most of the implants attracted a few melanophores. These were found usually on the surface of the tumor. Occasionally some of them migrated into the tumor mass and were found between the tubules.

During development from the 50 mm. stage to the beginning of metamorphosis (60 to 65 mm.) most of the tumors regressed (Table III and Fig. 9). The sharp outline of the tumor was gradually lost and replaced by a fuzzy, indistinct border which merged with the surrounding mesenchyme. The body of the





Illustrating the maximum amount of growth of carcinoma implants in the dorsal mesenchyme of the tadpole tail. Mag. × 5.5.

FIG. 7.—Four-day implant in 19 mm. host ($4AKT_{15}$).

FIG. 8.—One hundred and thirty-day implant in 55 mm. host ($4AKT_{2}$). See Fig. 11 for section of this tumor.

tumor gradually lost its dense whitish appearance and became more transparent. In more advanced stages of regression the implant could not be distinguished from the mesenchyme although the site could always be located by the greater density of the mesenchyme and by the presence of melanophores.

Most of the tadpoles bearing tail tumors were allowed to develop until they were 50 mm. or more in length. They were then autopsied. The implant was measured and preserved. Other parts of the animal were examined with special attention being paid to the kidneys but no tumors were found.

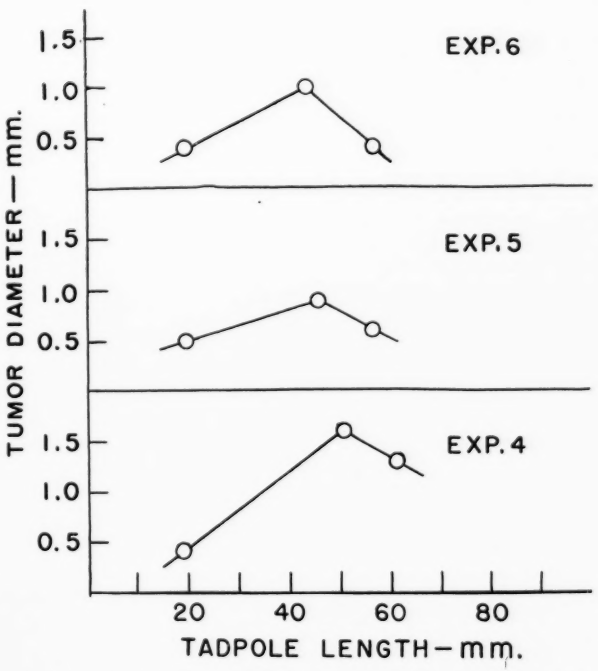


FIG. 9.—Graphs illustrating growth of adenocarcinoma implants in the tails of young tadpoles and regression during pre-metamorphic stages of development. Data from Table III.

Observations on the histology of tail implants.—Twenty-three of the successful carcinoma implants were sectioned serially. We have already noted from observations of the implants in living hosts that growth of the implant began when the tadpoles were about 35 to 40 mm. long and had carried the carcinoma tissue for about 1 month. Growth continued until the animals attained a length of about 50 mm. Thereafter there was apparent regression in most cases. The sectioned material shows that these gross observations were on the whole correct. Typical active tumors were found in young tadpoles while those in older hosts (50 mm. or more) were, with one exception, in various stages of regression. For the purposes of more detailed description the tail tumors have been divided into groups according to the histological picture they present.

Group 1 (4 cases—$5AKT_5$, 27 mm., 19 days; $5AKT_6$, 34 mm., 25 days; $5AKT_{10}$, 40 mm., 37 days; $4AKT_6$, 64 mm., 136 days): Typical growing adenocarcinoma. In three of these cases the hosts were young tadpoles. Sections show that the tumors they carried are composed of a small number of typical, actively growing carcinoma tubules. The stroma is sparse and there is no significant accumulation of mesenchyme cells or other cell types (Fig. 10). These cases suggest that the carcinoma implants assume tubular or cystic form in the earliest phases of growth in the tail. The remaining tumor of this group was taken from the tail of a 64 mm. tadpole. The histology is that of typical active kidney carcinoma, the main mass of the tumor consisting of closely packed tubules with little stroma between them. Mitotic figures are quite numerous. This is the only case in which an actively growing tumor was found in the tail of a large tadpole.

Group 2 (2 cases—$4AKT_2$, 56 mm., 138 days; $7AKT_{18}$, 55 mm., 64 days). The tumors have retained the characteristic structure but show an increase in the amount of stroma. Mitotic figures are rare and there are occasional pycnotic nuclei in the tubules (Figs. 11, 14).

Group 3 (4 cases—$4AKT_7$, 59 mm., 138 days; $4AKT_9$, 60 mm., 104 days; $4AKT_{11}$, 59 mm., 134 days; $7AKT_{19}$, 54 mm., 62 days). The tumor tubules are in various stages of regression ranging from intact ones containing a few mitotic figures to completely involuted ones (Figs. 12, 13). In general the epithelium of persisting tubules is reduced in height and contains some cells with pycnotic nuclei and others with a single large vacuole in the cytoplasm. The stroma is very dense and consists of spindle-shaped cells arranged concentrically around the individual tubules and around the tumor mass as a whole (Fig. 15). Tubules in more advanced stages of regression display marked reduction in the height of the epithelium and an increase in the number of pycnotic nuclei and vacuolated cells. In some sections the flattened epithelium can be seen to merge with the surrounding stroma which now contains a few vacuolated cells very similar to those in the tubules themselves (Fig. 16). Along with the regressing but recognizable tubules there are evidences of other completely involuted tubules (Fig. 13) These are represented by spindle cells and scattered vacuolated cells arranged concentrically around a lumen containing cellular debris. Some regressed tubules contain a central mass made up largely of vacuolated cells and surrounded by a layer of spindle cells. There is no tubular epithelium.

Group 4 (3 cases—$4AKT_4$, 60 mm., 138 days; $6AKT_5$, 60 mm., 72 days; $7AKT_{17}$, 59 mm., 63 days). In these cases the tumor epithelium has disappeared completely. The central part of the regressing tumor is made up almost entirely of vacuolated cells in concentric arrangement. Outside this central portion there is a layer of spindle cells. A few melanophores adhere to the outer surface of the mass (Fig. 17).

Group 5 (4 cases—$5AKT_2$, 65 mm., 58 days; $6AKT_1$, 53 mm., 64 days; $7AKT_8$, 62 mm., 63 days; $8AKT_1$, 65 mm., 56 days). All of the tumor tubules in each of these implants are completely involuted and replaced by dense masses of cells. These cells are commonly spindle-shaped, but may assume various forms and sizes. Many contain pycnotic nuclei. A few vacuolated cells are present. In more advanced stages of regression the small dense mass of cells representing the tumor is separated by a relatively clear area from the layer of melanophores which ordinarily adheres closely to the surface of implants (Fig. 18).

Group 6 (4 cases—$4AKT_3$, 59 mm., 134 days; $7AKT_3$, 58 mm., 102 days; $7AKT_6$, 61 mm., 88 days; $7AKT_{16}$, 50 mm., 103 days). These implants have undergone almost complete

regression. The site is occupied by slightly thickened mesenchyme containing scattered melanophores which previously were attracted around the tumor. In two cases a small central area consists of a few spindle-shaped cells and vacuolated cells in concentric arrangement. In one case the implant is represented only by a few cells with pycnotic nuclei and eosinophilic cytoplasm. These have no special arrangement. Finally in one case the implant has completely disappeared and the site is occupied only by slightly thickened mesenchyme containing a few melanophores.

Group 7 (2 cases—$7AKT_{13}$, 40 mm.(?), 22 days; $4AKT_1$, 71 mm., 136 days). The regressed tumor is replaced by large numbers of small round cells instead of by spindle cells and vacuolated cells as described above. In one case ($7AKT_{13}$) the center of the implant is occupied by a vesicle the inner wall of which appears to be made up of tumor cells. These have the typical large oval nuclei but are losing their columnar form, becoming oval or circular in cross section. As the cells round up they appear to break away from the epithelium and enter the vesicle. Surrounding the vesicle there are large numbers of small round lymphocyte-like cells. In the second case ($4AKT_1$) the site is occupied by a spherical dense mass of cells with no lumen or vesicle. This mass is divided into two parts. The central part consists mainly of small round cells with a few much larger pale, oval nuclei interspersed. These latter may be derived from the tumor epithelium. A collagenous capsule separates the central from the peripheral portions. The peripheral portion consists of a layer of densely packed small round cells without any of the larger pale nuclei. In these two cases it appears as if regression might have been brought about by lymphocytic infiltration.

Control series (69 cases).—Tadpole hosts received implants of normal kidney tissue in the mesenchyme of the dorsal tail fin. All of these were gradually resorbed. During the first month the yellowish kidney tissue was reduced in size. The borders of the implant became translucent and merged with the surrounding mesenchyme. During the second month following implantation resorption was completed, the implant in most cases being completely replaced by slightly thickened mesenchyme containing a few melanophores congregated at the site previously occupied by the kidney tissue. In a few cases a small core of dense yellowish tissue persisted at the implantation site.

Serial sections of ten representative implants were studied. These showed the implantation site to be occupied only by slightly thickened mesenchyme containing a few melanophores. At the very center there was usually a minute area composed of a few scattered nuclei in an abundant hyaline cytoplasm.

Implants Adjacent to the Kidney

Originally we intended to implant carcinoma tissue directly into the kidney substance, but in a few trial operations there was profuse bleeding following any attempt to make an incision in this organ. In lieu of direct implantation the carcinoma tissue was placed adjacent to the kidney in the following manner: An incision was made through the body wall in a position antero-dorsal to the right hind limb bud and adjacent to the postero-dorsal border of the right kidney (25 to 31 mm. tadpoles). With a small smooth glass rod the kidney was pried away from the trunk musculature for a short distance in this region and the implant inserted in the crevice so formed. The wound healed completely, usually within a day, and the tadpoles survived the operation quite well. There were only two postoperational deaths in a total of 49 operated tadpoles.

Experimental series (25 cases).—Two host tadpoles became moribund during the 4th week of the experiment. One ($5AKk_4$) was autopsied on the 25th day (27 mm.) while still living. At the implantation site we found an ivory-white tumor of circular, lobulated form. It was approximately 0.6 mm. in diameter (the diameter of the original implant was 0.25 mm.) and firmly attached to both the kidney and the trunk musculature. Over part of its lateral surface it adhered also to the body wall. In section the tumor consists of a small number of typical carcinoma tubules with numerous mitotic figures. There was no invasion of the kidney proper.

The second moribund tadpole ($5AKk_7$) was autopsied after death on the 26th day (31 mm.). The implant was found at the original site. Its diameter had increased from approximately 0.25 mm. to 0.4 mm. The relationship to surrounding structures and the microscopic appearance of this growth is the same as that described above for host $5AKk_4$.

The remaining 23 hosts carrying carcinoma implants adjacent to the kidney were allowed to develop for 82 to 150 days. Twelve were autopsied before metamorphosis—there were no tumors adjacent to the kidney, within the kidney, or elsewhere in the body. Eleven hosts were autopsied after completion of metamorphosis. There were no tumors at the

DESCRIPTION OF FIGURES 10 TO 13

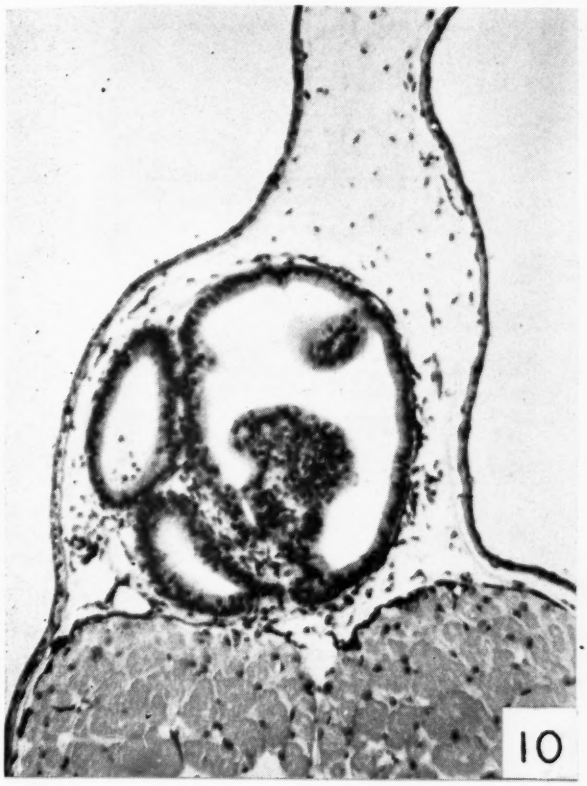
Growth and regression of carcinoma implants in the tadpole tail.

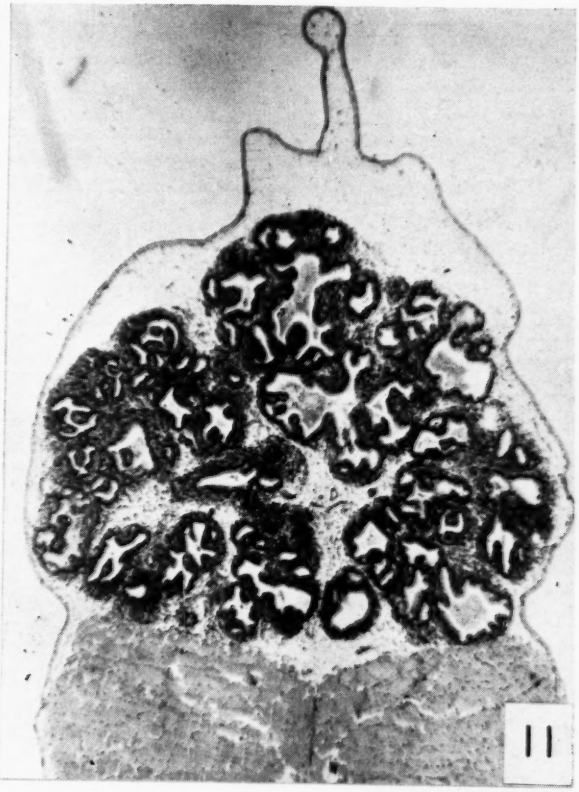
Fig. 10.—Early stage in growth of implant (host $5AKT_8$, 34 mm., 25 days after implantation). Mag. × 117.

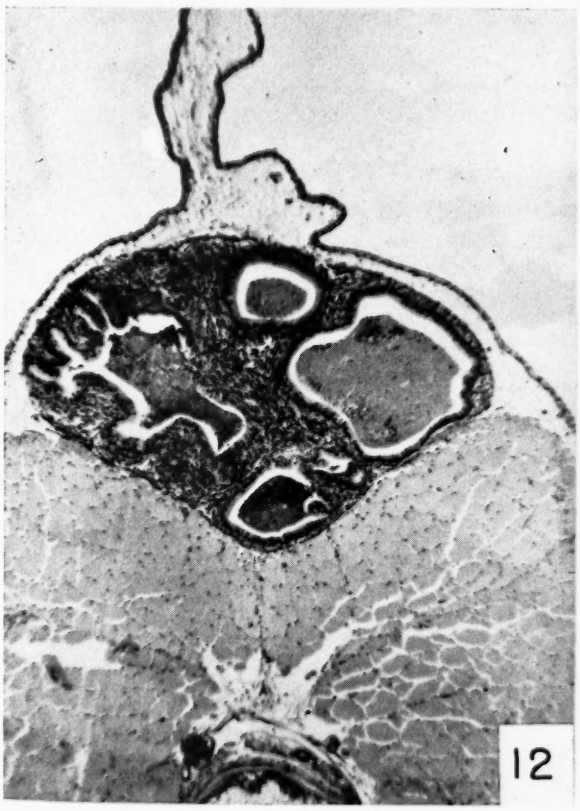
Fig. 11.—Maximum growth of implant. Beginning of increase in amount of stroma (host $4AKT_2$, 55 mm., 130 days). Mag. × 26.

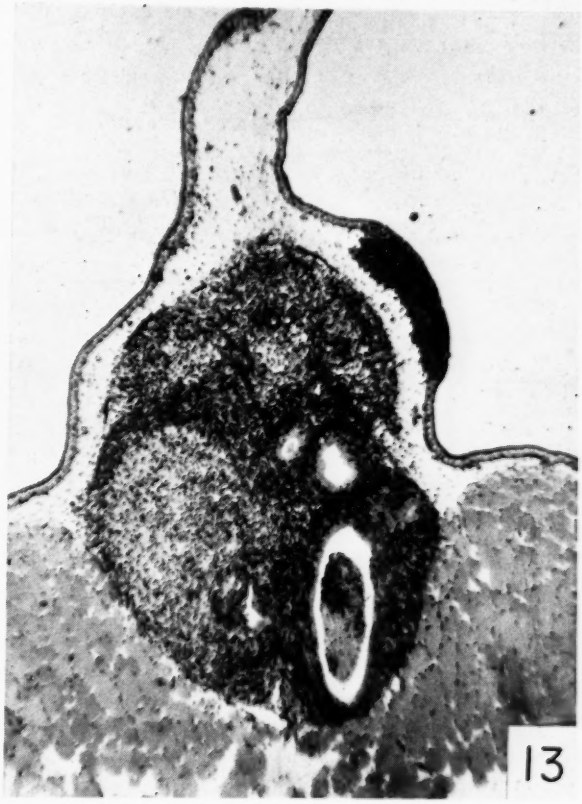
Fig. 12.—Regressing implant. Reduction in number of tubules, increase in stroma (host $4AKT_9$, 60 mm., 104 days). Mag. × 43.

Fig. 13.—More advanced stage in regression. More than half of the tumor tubules are involuted (host $4AKT_7$, 59 mm., 138 days). Mag. × 43.

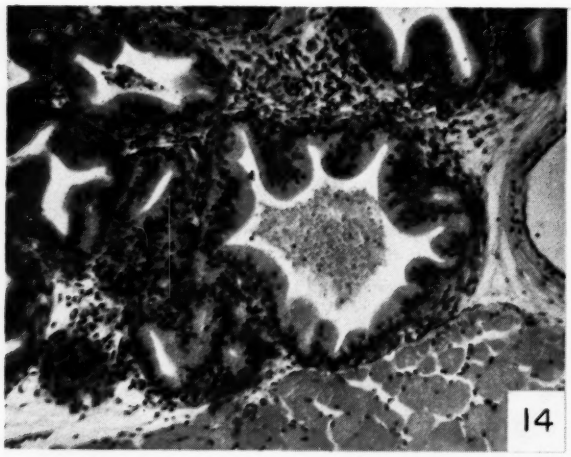


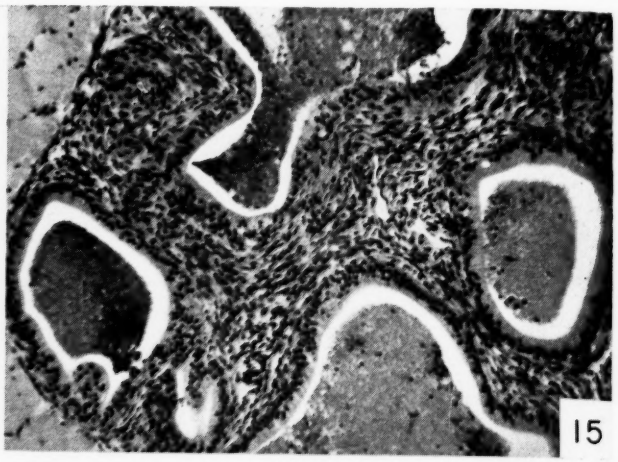


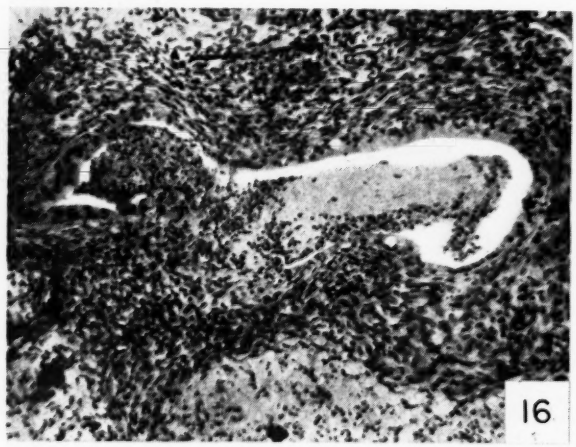


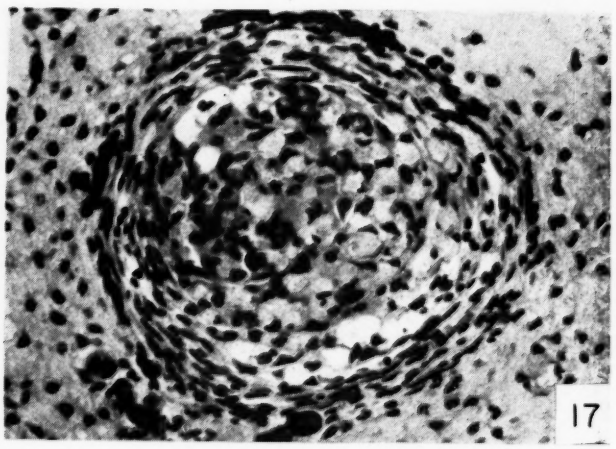


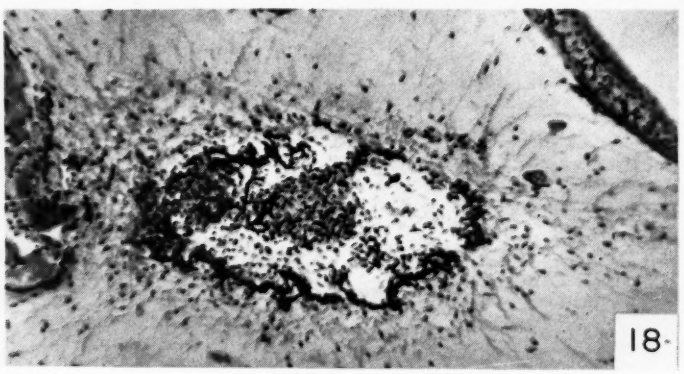
FIGS. 10-13











Histological changes of implanted carcinoma during regression in tadpole tail.

FIG. 14.—Typical tumor tubules, moderate stroma. (High power of Fig. 11.) Mag. × 86.

FIG. 15.—Reduction in height of tubular epithelium, loss of papillary projections into lumens of tubules, occasional vacuolated cells and cells with pycnotic nuclei. Dense stroma. (High power of Fig. 12.) Mag. × 86.

FIG. 16.—Tubular epithelium of tumor markedly reduced in height, merging with surrounding stroma. Increase in number of vacuolated cells and cells with pycnotic nuclei (host 4AKT$_7$, 59 mm., 138 days). Mag. × 86.

FIG. 17.—Late stage in regression. Note vacuolated cells in center surrounded by spindle cells. Melanophores are collected at outer surface (6AKT$_5$, 60 mm., 72 days). Mag. × 215.

FIG. 18.—Late stage in regression. Remnant of implant at center has contracted leaving melanophores separated from it by the clear area (host 8AKT$_1$, 65 mm., 56 days). Mag. × 86.

original site adjacent to the kidney, but multiple tumor loci were found in the kidneys of 3 hosts. These cases are of sufficient interest to be described individually.

Case 3AKk$_3$, 21 mm. frog, autopsied 85 days after beginning of experiment. In this host there was no tumor at the original site adjacent to the right kidney. The right kidney was also free of tumors, but in the posterior third of the left kidney there was a spherical tumor located ventro-laterally. It was 0.45 mm. in diameter, grayish-white in color, of firmer consistency than the surrounding kidney tissue, and composed of large, closely packed tubules. The anterior two-thirds of the kidney containing a part of this growth was serially sectioned. Histological examination confirmed that the growth was typical kidney carcinoma. The sections also revealed another completely separate tumor locus in the center of the anterior third of the same kidney. It consists of a single tumor tubule sharply marked off from surrounding normal kidney tubules. The walls of the tubule are pseudostratified columnar epithelium; individual cells are larger than normal kidney tubule cells and stain more lightly with both hematoxylin and orange G. Mitotic figures are very numerous. Perhaps the most interesting feature of this tumor tubule is that it is structurally continuous with a normal kidney tubule (Fig. 21). The same type of connection between normal and tumor tubules was found in other kidneys (see below); the following descriptive account holds for all of these cases. The cells of a normal kidney tubule located at a distance from the tumor or having no connection with it are cuboidal, with cytoplasm which stains deeply with orange G, and nuclei which are circular in section and which contain coarse chromatin granules densely stained with hematoxylin. Cells making up the part of a normal tubule near the point of juncture with a tumor tubule maintain the cuboidal shape but are often slightly enlarged. The cytoplasm of these cells stains more lightly with orange G and nuclei contain chromatin granules that are smaller and more dispersed than the granules of unaltered nuclei. Immediately adjacent to the transition point the normal epithelium becomes two or three cells thick and thus approaches the thickness of the carcinoma epithelium. The tall columnar tumor cells bordering on the "normal" epithelium are very distinctive both in size and staining properties. The height of these cells is 2 to 3 times that of the normal cells. The cytoplasm is light purple with a slight reddish or yellowish tinge; nuclei are large, oval, and contain finely dispersed chromatin granules. In the material studied there were no cell types intermediate between the slightly modified normal kidney tubule cell described above, and the full-fledged carcinoma cell.

Case 3AKk$_4$, 22 mm. frog, autopsied 85 days after implantation. Both kidneys of this animal contained multiple tumor loci varying from 0.2 to 0.7 mm. in diameter. The right kidney and the anterior half of the left kidney were serially sectioned. Microscopic examination revealed the following features: In the anterior half of the left kidney there are five separate loci. The largest of these occupies roughly half the cross-sectional area of the kidney and consists of one large tubule. Part of this tumor tubule contains a sizable cyst with a few papilliform projections of the tumor epithelium extending into it (Fig. 22). The other four loci are smaller. All consist of unmistakably typical tumor epithelium sharply contrasting with the normal epithelium of the adjacent kidney tubules. One of these smaller loci is structurally continuous with a normal kidney tubule. All loci are very active mitotically.

The right kidney contains 10 distinct and separate loci. The largest of these is a cystic tumor, about 0.5 mm. in diameter, and located in the postero-lateral region adjacent to the original implantation site. However, there are no tumors outside the kidney at the actual site. Two other noncystic growths of about the same size are located in the central part of the kidney in this region. The remaining loci are smaller and distributed through the middle and anterior part of the kidney. Four of the smaller tumors each has a connection with a normal kidney tubule similar to the connections already described. All of the loci have the microscopic structure of the adult kidney carcinoma except that there is no stroma in the very early stages of the growth of a tumor locus. Again, mitotic figures are very numerous.

Case 3AKk$_5$, 22 mm. frog, autopsied 86 days after implantation. The anterior half of the right kidney contains four separate loci with diameters ranging from 0.15 to 0.5 mm. Each of these consists of a single typical tumor tubule; one of these tubules is structurally continuous with a normal kidney tubule.

Control series (24 cases).—Control tadpoles carrying normal kidney tissue implants were reared under the same conditions and autopsied at the same times as were the experimental hosts. The implants were all resorbed and no tumors were found.

Implants to the Liver

Adult kidney carcinoma tissue was implanted directly into the liver. In performing this operation an incision was made in the ventro-lateral body wall adjacent to the liver in 24 to 33 mm. tadpoles. By applying gentle pressure to the body wall in this area the median or the right lobe of the liver could be forced outward through the incision. A slit was then made in the liver with a small corneal knife, the implant inserted, and the lobe of the liver replaced in the body cavity. Bleeding was slight and the body wall incision healed within a day or two. Only one animal of a total of 42 died as a result of this operation.

Experimental series (19 cases).—The tadpoles developed satisfactorily and were autopsied 85 to 150 days after receiving the implant. At autopsy all organs were examined carefully under the dissecting microscope. The livers were cut into small pieces and each piece scrutinized for the presence of tumor tissue.

In one host a large tumor developed in the liver. This tadpole (5AKL$_3$) had carried the carcinoma implant for 124 days. At the end of this period its total length was 46 mm. and its body length (tip of snout to anus) 17 mm. The tumor this animal carried measured 8 mm. from left to right, 7.5 mm. from anterior to posterior, and 6.5 mm. from dorsal to ventral. Assuming a spherical shape with a diameter of 7 mm. the volume would be roughly 180 mm.^{3} The volume of the original implant was approximately 0.008 mm.^{3} (diameter = 0.25 mm.). Thus the mass of the tumor increased 22,500 times.

The external appearance of this tumor was very much the same as that of a large kidney tumor in an adult frog (Fig. 19). It was smooth-surfaced, of firm consistency, and ivory-white in color. Apparently the

median lobe of the tadpole's liver was almost completely replaced; only a remnant of it remained on the ventral surface of the tumor mass. The two other liver lobes were intact as were other organs. There were no metastases. The kidneys were sectioned serially but revealed no growths.

A portion of this growth was sectioned. Its histology is that of a typical active kidney adenocarcinoma (Fig. 20). The main part consists of tumor tubules and acini; stroma is rather sparse. There is one cyst containing papillary extensions of tumor epithelium. Mitotic figures are fairly numerous.

Nine of the remaining 18 hosts were autopsied in tadpole stages. There were no tumors in the liver, kidney, or elsewhere in the body. Finally, 9 hosts were autopsied after completion of metamorphosis. In 7 cases the liver, kidneys, and other organs were free of tumors. In 2 cases tumors were found in the kidneys although the original implant in the liver had failed to grow and was not found at autopsy. In both of these hosts the gross and microscopic appearance of the kidney tumors is very much the same as that of the tumors in the kidneys of animals which received implants adjacent to the kidney (see preceding section). In animal $3AKL_4$ (21 mm. frog, 82 days postoperation) the left kidney contains eight separate loci with diameters ranging from 0.1 to 0.4 mm. approximately. The smaller loci are each composed of a single typical tumor tubule. Four of the loci show structural continuity between tumor tubule and a normal kidney tubule, the transition between the two types of tissue being abrupt (Fig. 21). Case $3AKL_2$ (22 mm. frog, 82 days) is very similar to the one just described. The right kidney contains 12 separate loci, each consisting of a single tumor tubule. The diameters of the different tubules vary from less than 0.1 mm. for small noncystic ones, to about 0.3 mm. for one relatively large tubule containing a cyst. At four of the loci there appears to be continuity between tumor tubule and normal kidney tubule of the type already described. Mitotic figures are very numerous in the tumor tissue.

Control series (23 cases).—Implants of normal kidney tissue were resorbed; no tumors were found at autopsy.

Implants to the Body Cavity

Implants of adult kidney carcinoma tissue were placed in the body cavity of hosts at two stages of development. In the younger hosts (experiment 2AKa, 9 to 10 mm., 14 cases) the implant was inserted through a slit in the antero-lateral body wall and then wedged between the splanchnic mesoderm of the gut and the somatic mesoderm of the body wall. In the older hosts (experiment 2AKb, 18 to 23 mm., 5 cases) the implant was inserted through a slit in the ventro-lateral body wall and came to lie in the vicinity of the posterior part of the kidney. Control tadpoles received implants of normal kidney tissue in the same sites. All animals survived the operation, developed satisfactorily, and were autopsied 70 to 90 days after the beginning of the experiment.

Experimental series (19 cases).—The results are summarized in Table I. In one tadpole ($2AKa_{14}$, 43 mm., 88 days) a spherical or slightly oval-shaped, smooth-surfaced, grayish-white tumor was found attached to the intestine by a short stalk of connective tissue containing blood vessels leading from the outer wall of the intestine to the tumor. The average diameter of the growth was approximately 1.4 mm. and the volume roughly 1.4 $mm.^3$ (Volume of original implant was 0.02 $mm.^3$) Sections of this growth reveal a histological structure identical with that of actively growing adult kidney adenocarcinoma. There is very little stroma, practically all of the growth being made up of the tumor tubules. Mitoses are numerous. There is no sign of invasion of the part of the intestine to which it is attached.

In the remaining 18 cases no tumors were found in the body cavity. One little frog ($2AKb_5$, 72 days) which died during final phases of metamorphosis was found on autopsy to have a tumor in the anterior half of the left kidney. Under the dissecting microscope it appeared grayish-white in color and composed of numerous large tubules. The dimensions of the growth were: length—1.3 mm., width—1.2 mm., thickness—0.6 mm. In this animal the dimensions of the kidney were 5 mm. × 1.4 mm. × 0.8 mm. Thus the tumor was relatively large. Sections of the tumor show the gross structure of adenocarcinoma, but finer

DESCRIPTION OF FIGURES 19 TO 22

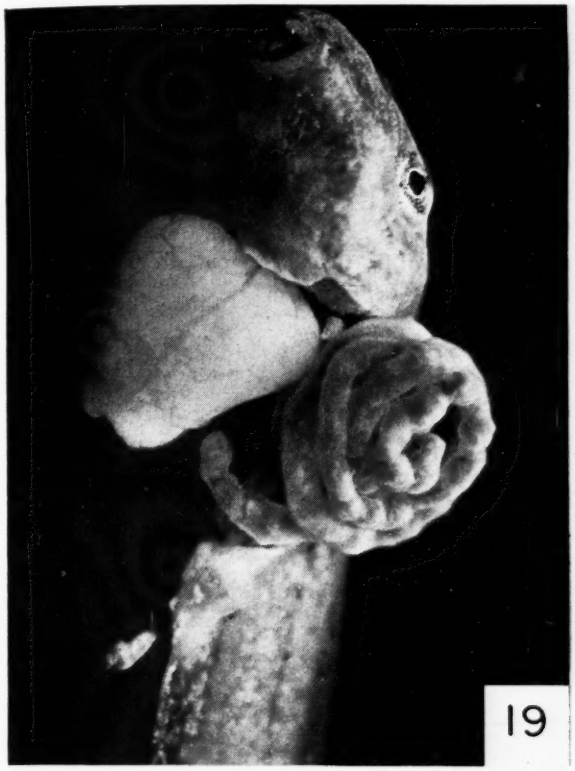
Tumors in the liver and kidney.

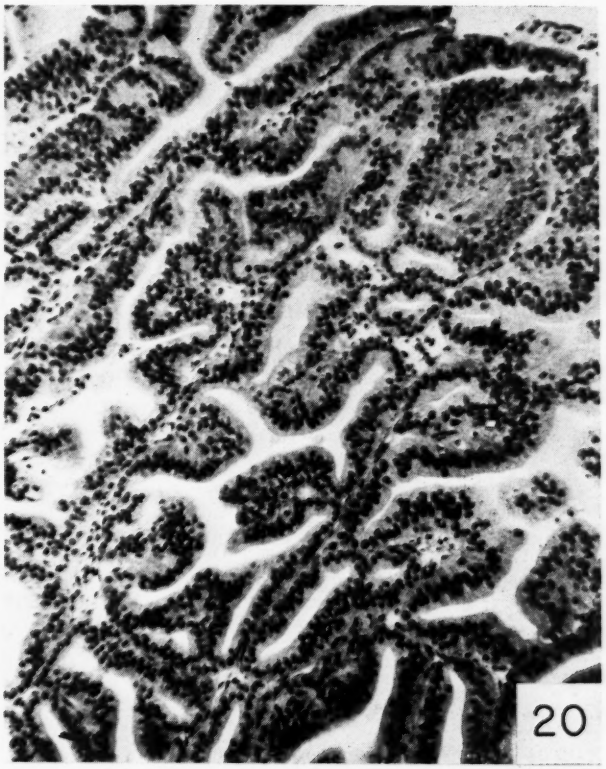
Fig. 19.—Large tumor developed from implant in median lobe of liver. The white mass is composed entirely of carcinoma tissue (host $5AKL_3$, 46 mm., 124 days). Mag. × 4.

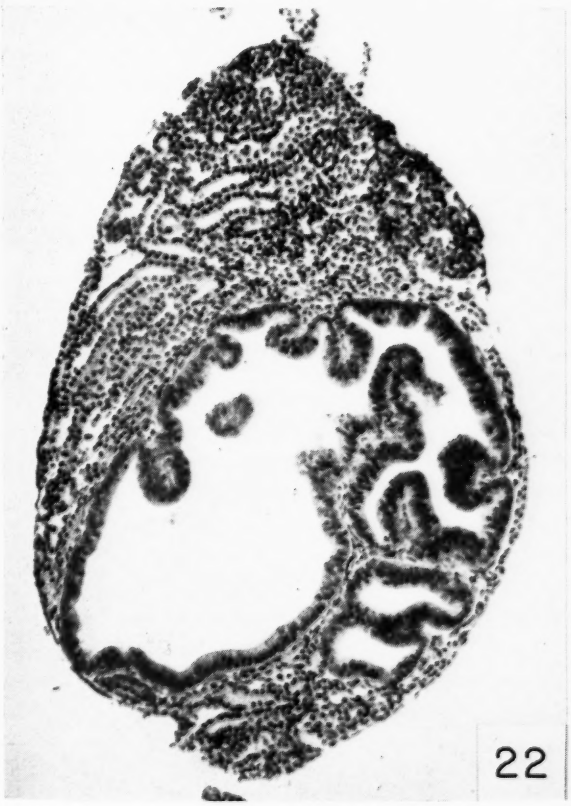
Fig. 20.—Section of a part of the tumor shown in Fig. 19. Mag. × 114.

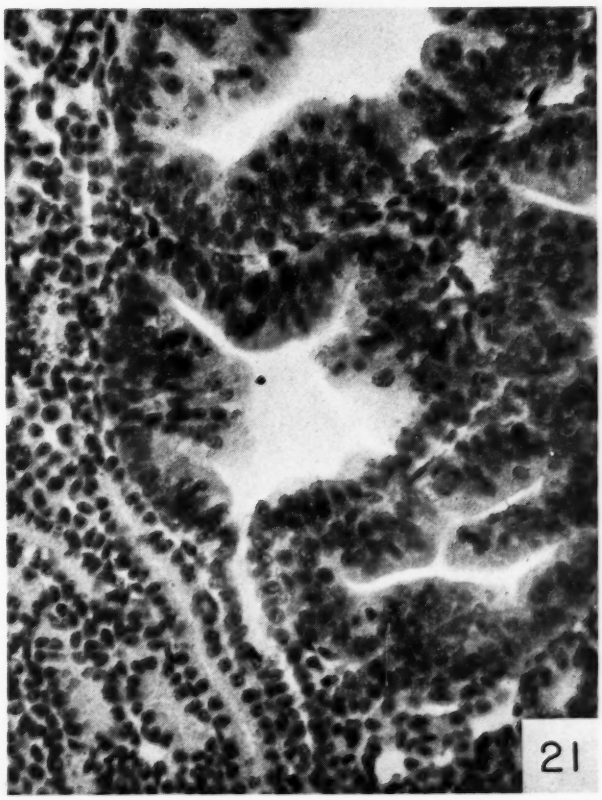
Fig. 21.—Tumor locus in the kidney. Original implant was made to the liver and did not take at that site. Lower part of tumor tubule in center of photograph is structurally continuous with a normal kidney tubule (host $3AKL_4$, 21 mm. frog, 82 days). Mag. × 285.

Fig. 22.—Tumor locus in the kidney. Original implant was made adjacent to the kidney and did not take at that site (host $5AKk_4$, 22 mm. frog, 85 days). Mag. × 114.









FIGS. 19-22

details are lacking due to the poor condition of the tissue at the time of fixation.

Control series (24 cases).—The implants of normal kidney tissue were resorbed. No tumors developed in the kidneys or elsewhere in the control hosts.

DISCUSSION

The frog kidney adenocarcinoma can be successfully transplanted to various sites in the tadpole where it assumes the same gross appearance and histological structure that it has in the adult. This demonstrates that there is nothing in the general larval environment which will inhibit the growth or alter the structure of this tumor. On the contrary, in some respects the larval host is more favorable than is the adult. Transplants in the adult will grow directly only in the anterior chamber of the eye; in the lymph sacs, cranial cavity, and abdomen transplanted material is resorbed (9, 11). In the tadpole minute implants grow into sizable tumors in all of the sites used. The greater success of implants in the larval host may be due simply to more rapid attachment and vascularization, or conceivably to some factor peculiar to the larval organism.

Lucké has shown for adult frogs that although transplanted material fails to produce a tumor at the original site (excluding the eye), it does result in an increased incidence of tumors in the kidney. Somewhat the same situation holds in tadpoles. Some implants to liver, body cavity, and adjacent to the kidney failed to grow at the original site but somehow produced tumors in the kidneys. How these renal loci arise presents an interesting question. It is possible that the implants metastasized to the kidney even though they failed to take at the original site. But it is more probable that the tumors were induced by a cell-free agent or virus. Lucké (9) has demonstrated the probable existence of an organ-specific virus which induces tumors in the kidneys of adult frogs. In our experiments the histology of the tumors in the kidneys of just-metamorphosed froglets suggests that they too are induced *in situ* and do not arise from metastatic emboli. The tumors are multiple with no connection between individual loci in the same kidney even though they may be numerous and relatively close together. Furthermore, many of the smaller loci consist of a single small tumor tubule which is structurally continuous with a normal kidney tubule, the transition between the two types of tissue being quite abrupt. A metastatic embolus would not be expected to form a structural unit with a normal kidney tubule. It is more probable that a part of the normal tubule was induced to transform into tumor tissue and that the structural connection between the two types of tissue was maintained during early stages of growth.

There is one other interesting point concerning the kidney tumors observed in these experiments. Although all implants were placed in the tadpoles at an early stage, no tumors were found within the kidneys until after the completion of metamorphosis. This raises the question as to whether the tadpole kidney is susceptible to the development of tumors. The data reported here are not extensive enough to answer this question, but we hope that future experiments will succeed in correlating differences in susceptibility with differences in structure and function between adult and larval kidneys.

We were not able to observe possible effects of metamorphosis on tumors in all of the implantation sites. As we have mentioned above, it appears that the kidney does not develop tumors until after completion of metamorphosis. Implants in the trunk subcutaneous tissue may go through the metamorphic period unaffected. It is impossible to say how metamorphosis would affect tumors in the body cavity and liver. There was but one of each of these and the hosts bearing them were autopsied while still in the tadpole stage.

The tail presents a special case. The power of regeneration, by which we may detect the presence of morphogenetic "fields," is greater in young tadpoles than in old ones (3, 14, 15, 21). Since carcinoma implants grow well in the tails of relatively young tadpoles it appears that the tail "field," if it is operating under the conditions of the experiment, has no restrictive or organizing effect on the tumor. Growth of tail implants ceases and regression begins during later stages of host development when the growth rate of the tail and its power of regeneration are reduced. The circulation of regressing tumors appears intact. Also, different tumors stop growing after having attained quite different sizes, which indicates that the physical factor of space available does not limit tumor growth in the tail. This leaves open two alternative interpretations of the regression of tail implants. They are: (a) that it is spontaneous, and (b) that the factor or factors responsible for the cessation of growth of the tail and its loss of regenerative power also result in cessation of growth and regression of the implanted tumors. Observations on the growth of tail implants in experimentally produced permanent tadpoles, and in tadpoles undergoing induced precocious metamorphosis, should indicate which of these interpretations is the correct one.

SUMMARY AND CONCLUSIONS

The kidney adenocarcinoma of the adult frog (*Rana pipiens*) has been transplanted to a total of 163 tad-

poles to determine if the larval environment has any effect on its structure and behavior. The implants developed into typical growing tumors in all of the following sites: 1. subcutaneous tissue of trunk (7 cases); 2. mesenchyme of dorsal tail fin (27 cases); 3. adjacent to the kidney (2 cases); 4. liver (1 case); and 5. body cavity (1 case). One hundred and eighty control tadpoles received implants of normal kidney tissue and developed no tumors.

Tumors in the trunk subcutaneous tissue grew well and in two cases survived metamorphosis unaltered.

Tumors in the tail ceased growth and regressed during later stages in the development of the host tadpoles. This regression occurred before the initiation of metamorphic absorption of the tail.

Typical tumors developed in a small number of cases from implants in the liver, body cavity, and adjacent to the kidney. The majority of implants in these sites were resorbed. Six of the hosts which failed to develop a tumor at the implantation site were found to have multiple tumor loci in the kidneys. These occurred only in hosts which had completed metamorphosis. Many of the individual tumor tubules making up the smaller loci showed structural continuity with normal kidney tubules.

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Abnormalities in the Distribution of Biotin in Certain Tumors and Embryo Tissues

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In a preliminary note (13) it was pointed out that certain tumors contained much more, others significantly less, vitamin H or biotin than did normal adult tissues. It was also observed that embryo tissues deviated from the normal biotin levels in the same manner as the corresponding tumors. Because of the indispensability of biotin for the growth of many microorganisms and higher animals it was considered possible that these differences might reflect fundamental metabolic changes characteristic of rapidly multiplying cells. Therefore, the earlier observations have been extended to include many more tumor types. In addition, various attempts have been made to alter the biotin content of tumors experimentally and thus influence their growth.

MATERIALS AND METHODS

Tissues were assayed for biotin by a new microbiological method using the indicator organism *Rhizobium trifolii* 205, for which biotin is a specific growth factor (12). Only a small proportion of the biotin in tissues is in free form (Table I), the remainder being firmly bound and liberated from such combination only after hydrolysis. Thus the treatment of the tissue to be assayed differed somewhat depending upon whether free or total biotin was to be determined.

For total biotin assays, about 0.3 to 0.5 gm. of tissue was extracted by autoclaving with 5 ml. of 10 per cent sulfuric acid for one hour at 15 pounds' pressure which freed the biotin from its combination in the tissues. The extract was then filtered into a calibrated tube, the solid debris washed, and washings added to the filtrate. After neutralizing the filtrate to bromthymol blue with 25 per cent sodium hydroxide it was diluted to 10 ml.

For free biotin assays, acid hydrolysis was of course not necessary. A similar amount of tissue was simply chopped very finely and extracted with 5 ml. of water by placing the tubes in a boiling water bath for 20 minutes. The mixture was then filtered, the residue washed as before, and the filtrate diluted to 5 or 10 ml.

The basal biotin-free culture medium for the test organisms consisted of the following: mannite 5 gm., dipotassium phosphate 0.5 gm., potassium nitrate 0.2 gm., magnesium sulfate 0.2 gm., sodium chloride 0.1 gm., calcium sulfate 0.1 gm., ferric chloride 0.01 gm., thiamin 100 γ, β-alanine 100 γ, distilled water 1 liter. The mixture was brought to boiling, filtered while hot, and on cooling adjusted if necessary to pH 6.8. This medium is incapable of supporting growth of the organism, but since the only additional factor required is biotin, growth occurs upon the addition of tissue extracts in proportion to the amount of biotin they contain.

Various dilutions of the tissue extract to be assayed were measured in amounts from 0.2 ml. to 0.001 ml. into 125 ml. Erlenmeyer flasks, and 25 ml. of basal medium then added to each. After autoclaving for 30 minutes at 15 pounds' pressure, these mixtures were inoculated with one loopful of a light suspension of bacteria in sterile distilled water (approximately 50 to 100 million cells of *Rhizobium* per ml.). The inoculum was grown on slant cultures consisting of basal medium plus agar 1.5 per cent and a small amount of biotin supplied in the form of 0.1 per cent Difco yeast extract. Insufficient biotin is carried in such an inoculum to permit growth in the basal medium.

These inoculated mixtures were incubated for 5 days at 28° C., after which the opacity resulting from bacterial growth was measured with the Evelyn photoelectric colorimeter, filter 540. From standard curves for this organism, readings were converted into numbers of *Rhizobium* per ml. and the results expressed graphically. By comparison with crystalline biotin or standardized yeast extract curves included in every assay, the amount of unknown extract inducing half-maximum growth was determined, and since this corresponds to the activity of 0.32 millimicrograms of biotin in 25 ml. of medium, the biotin content of the extract could be readily calculated. All results are expressed in mγ (1×10^{-9} gm.) of biotin per gm. of dried tissue.

The Biotin Content of Tumor, Embryo, and Normal Adult Tissues

In this comparative study of the biotin contents of normal and malignant tissues considerable care was exercised in the selection of material. Only those tumors for which suitable control tissues could be obtained were chosen. Samples of each were examined microscopically and unless found to be quite healthy and reasonably free from other tissues, the material was discarded. It is obvious that these restrictions must have eliminated at once a considerable proportion of all available human material and much animal material as well. For example, many of the commonest tumors, such as carcinoma of the breast or uterus, were unsuitable because a control sample of the corresponding normal epithelium was not available. Likewise carcinomas of the stomach or intestine were usually valueless, either because of necrosis in the tumor itself or of inability to obtain normal control epithelium free from underlying tissues. The results which are reported below, therefore, represent as nearly as possible a true comparison of the vitamin H contents of certain normal tissues and their malignant derivatives.

Skin tumors.—Many specimens of the Shope rabbit papilloma and related carcinomas were generously supplied for this work by Dr. John G. Kidd, of the Rockefeller Institute for Medical Research. By careful dissection it is possible to obtain rabbit skin relatively free from the subjacent connective tissue, so that by microscopic comparison there is an equally high proportion of epithelial tissue in both normal skin and tumor. Results of biotin assays (Fig. 1) indicated an average of 360 mγ of biotin per gm. of tissue for 5 papillomas, while 16 skin carcinomas averaged 369 mγ. In contrast to this, four samples of normal skin taken from areas adjoining the papillomas contained only 89 mγ of biotin per gm. Since certain similarities in the carbohydrate metabolism of embryonic and tumor tissues are known to exist, determinations of biotin in the former were included for comparative purposes whenever possible. The skins of domestic rabbit fetuses approximately 3 days before birth contained an average of 531 mγ of biotin per gm. From these assays it is apparent that although skin papillomas and carcinomas of the rabbit do not differ significantly in biotin content, they are both far richer in the vitamin than the corresponding normal adult tissue, while the value for embryo rabbit skin is still higher.

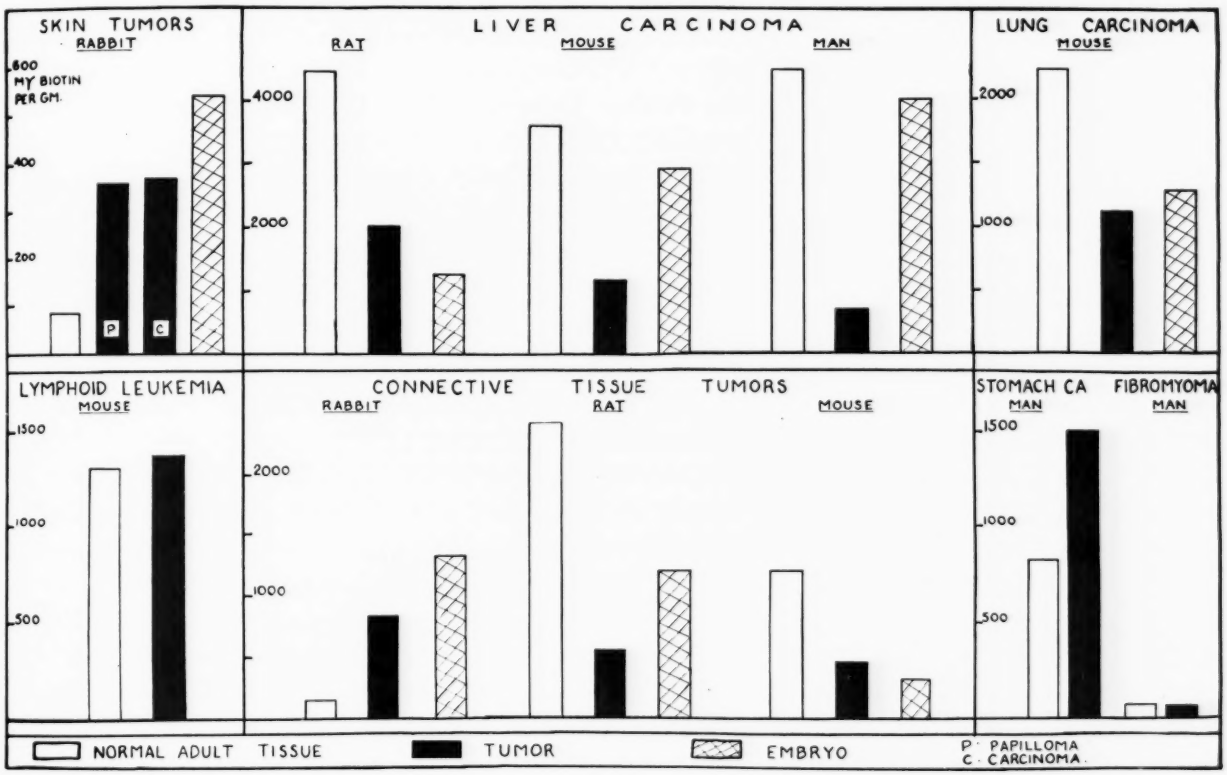

Fig. 1.—A comparison of the biotin contents of certain tumors with analogous normal adult and embryo tissues.

Liver tumors.—Primary tumors of the liver, when found to be well circumscribed and free from necrosis and connective tissue, were considered ideal for biochemical study, since an adequate supply of normal liver was readily available from the same animal for comparison. Such tumors were produced experi-

mentally in Wistar rats by feeding a diet of unpolished rice plus 0.06 per cent *p*-dimethylaminoazobenzene (butter yellow) over a period of 3 to 6 months. Several suitable tumors were obtained, 6 of which on analysis were found to contain an average of 2030 mγ of biotin per gm. (Fig. 1). Some had considerably less biotin than this, but were eliminated because histological study showed that they contained excessive amounts of connective tissue. Parts of the tumor-bearing livers which were shown microscopically to be free of cirrhosis as well as of tumor, were used as controls, and averaged 4480 mγ biotin per gm., a value almost identical with those obtained for the livers of normal nonpregnant adult Wistar rats on the standard laboratory ration of equal parts Purina dog chow and Rockland mouse or rat diet, with water ad libitum. Embryo rat liver taken on the 17th to 18th day of gestation possessed only 1280 mγ of biotin per gm. Here again, therefore, embryo and tumor tissue were found to differ in their biotin content from that of the normal adult tissue, although in this instance the deviation was in the opposite direction from that of the skin tumors previously discussed.

The close parallelism between the observed abnormalities in biotin level of embryo and tumor suggested that perhaps the difference in vitamin H content is related to changes normally occurring in all rapidly dividing cells rather than to any metabolic peculiarity of those particular tissues. To clarify this point, rats were partially hepatectomized, and the vigorously regenerating livers assayed for biotin 3, 7, 14, and 21 days after the operation. Even during the most active phase of regeneration, which occurred in from 3 days to one week, there was no significant change in the biotin levels, 12 regenerating livers averaging 4780 mγ per gm. (range 4300 to 5720).

Similar determinations were made on a number of subcutaneously transplanted liver carcinomas of mice, which were generously supplied from the National Cancer Institute by Dr. Howard B. Andervont. The growth was originally induced by subcutaneous injections of butter yellow. It was surprising to find that in four 28-day tumors of moderate size (0.6 gm. or less) the biotin level was 3832 mγ per gm. (range 3130 to 4216), practically identical with that of the control livers (from the same animals), which contained 3563 mγ. The biotin levels of these livers did not differ from those of normal animals of the same strain. On assaying the carcinoma at a later stage of growth (over 1 gm.) it was found that while 6 normal livers from tumor animals averaged 3613 mγ of biotin per gm., the average biotin level of nine 34-day tumors was but 1170 mγ (range 555 to 2130). For comparative purposes, it may be stated that the biotin level of mouse embryo liver has been found to be 2909 mγ (Fig. 1).

While these analyses of older tumors indicate changes in biotin content similar to those observed in carcinoma of the rat liver, the reason for the unchanged biotin levels of the younger mouse tumors is obscure. This discrepancy between two values for the same tumor strain has also been noted recently with the transplanted liver tumors of C3H mice, also supplied through the kindness of Doctor Andervont.

A few primary liver cell carcinomas of human beings have been assayed for biotin but there was only one from which ideal material could be obtained and for which reliable figures may be reported. This appeared as a large, firm, light yellowish-brown mass in the left lobe of the liver with several large metastases throughout the rest of the organ. The tumor contained very active tissue practically free of necrosis, while the unaffected liver appeared quite normal, with no evidence of cirrhotic change. The normal liver assayed 4450 mγ of biotin per gm., and in striking contrast to this the carcinoma was found to contain only 746 mγ per gm. For purposes of comparison, values for human embryo organs obtained from a 4½-month fetus, indicated the biotin level of human embryo liver to be 4000 mγ per gm., which, unlike that for embryo rat livers, is only slightly below adult values (Fig. 1).

Lung tumors.—Several strain A mice bearing the transplanted lung tumor F were generously supplied by Doctor Andervont. This carcinoma arose spontaneously and its source is thought to have been the alveolar epithelium. If so, the normal lung tissue selected as control should provide a fair, though admittedly imperfect, basis for comparison. As indicated in Fig. 1, normal adult mouse lung contained an average of 2285 mγ of biotin per gm., while the carcinomas were all much lower (1177 mγ). Samples of mouse embryo lung obtained near term averaged 1330 mγ of biotin, a deviation from the adult value which closely approximates that of the tumors.

Connective tissue tumors.—Several types of transplantable sarcomas were readily available in experimental animals, and as controls normal connective tissue scraped from beneath the epithelium of adult mice was employed. This assayed 1280 mγ of biotin per gm., whereas the transplanted mouse sarcomas 37 and 180 averaged 556 mγ and 455 mγ, respectively (Fig. 1). The biotin content of the corresponding embryo tissue proved to be 312 mγ per gm., a finding which again bears out the observation that tumor and embryo appear to deviate in similar fashion from the normal adult tissue.

Adult rat connective tissue contained an average of 2460 mγ of biotin per gm. While, as in the mouse, samples of connective tissue were taken from subcutaneous areas apparently free from gross fat deposits it was nevertheless impossible to obtain the tissue entirely devoid of fat cells. Fat is very low

in biotin, however (52 mγ per gm. for the rat) so that the small amounts present in the control would lower the biotin level somewhat from its true value. The R39 rat sarcoma was found to contain 544 mγ of biotin per gm. and rat embryo connective tissue 1280 mγ (Fig. 1).

Samples of two different transplantable rabbit sarcomas, RS and Kato, were obtained through the courtesy of Doctor Kidd. These assayed 290 and 813 mγ biotin per gm., respectively. In contrast to the results with normal adult rat and mouse connective tissue, the same tissue in the rabbit assayed only 134 mγ. Embryo rabbit connective tissue from fetuses approximately 3 days before birth averaged 1330 mγ (Fig. 1). The increased biotin content of the rabbit sarcomas over those of the control adult tissues duplicates the findings for epithelial growths in this species, and the two are contrary to those generally found for rat and mouse tumors. In either case, however, the corresponding embryo tissues consistently varied from the normal biotin levels in parallel with the tumors.

Lymphoid leukemia.—Because of the resemblances between the leukemias and neoplasia, the biotin levels of normal lymph nodes were compared with the enlarged nodes of lymphoid leukemia. For this purpose Dr. Jacob Furth, of Cornell University Medical College, kindly supplied us with a number of mice that had been inoculated with his line AKh 1032. Several groups of leukemic nodes and normal nodes from uninoculated mice of the same and different strains were examined. The results were uniform, the normal nodes averaging 1307 mγ (range 1300 to 1330), the leukemic nodes 1390 mγ (range 1310 to 1405) biotin per gm., an insignificant difference. Thus these nodes did not exhibit the variation found in most neoplasms, but as those of other lines might, no conclusions can yet be drawn.

Miscellaneous tumors of human origin.—Nearly all human material obtained either at autopsy or at operation proved to be unsatisfactory in one way or another. Many bone sarcomas were assayed for biotin value, but here the mass of intercellular material, even when inorganic constituents were eliminated, made comparisons of normal tissue and tumor extremely difficult to evaluate. Results on several bronchogenic carcinomas of the lung, although the tumors were ideal in themselves, were discarded because alveolar tissue is really not an adequate control and attempts were not made to assay bronchial epithelium. Most carcinomas of the gastrointestinal tract were highly unsatisfactory either because of necrosis or hemorrhage or excessive connective tissue in the tumor, or the presence of too much connective tissue or muscle in the sample of normal mucosa selected as a control. A few, however, did prove to be acceptable following microscopic verification of the tissues assayed. The values for the tumors in each of these instances differed widely from the control determinations. Carcinomas of the stomach, sigmoid, and rectum possessed an increased biotin content, 1500, 787, and 444 mγ per gm., while the respective control values were 833, 284, and 102 mγ. In two cases, however, a reverse relationship was discovered, the biotin levels of carcinomas of stomach and cecum being 483 and 1140 mγ while corresponding control determinations were 1700 and 2250 mγ per gm. (Fig. 1). As one would expect, the results of assays on isolated human tumors, each with its own clinical and pathological peculiarities, do not present the uniform picture which is obtained in the case of a group of standard animals bearing tumors of common origin. It is not surprising, in such a random selection of material from patients, that tumors of both the biotin-rich and biotin-poor type were encountered, for even in the normal tissues a wide divergence in biotin concentration was found in different patients, probably because of their variable states of nutrition. In cases of accidental death in healthy subjects, the biotin levels of the organs were remarkably constant.

As for benign growths of the human subject, a large lipoma in the posterior cervical region and 4 uterine fibromyomas have been examined so far. None contained more biotin than the mother tissue. Thus for the lipoma the value was 35 mγ as compared with 69 mγ for normal adult fat. The fibroids, all of which were obtained at operation, gave such uniform figures that they may safely be regarded as characteristic of this lesion (Fig. 1). The average was 67 mγ and that for the surrounding normal myometrium 70 mγ per gm.

The uterine fibroid is considered by some to be a hyperplasia rather than a true neoplasm, and if this view be correct a normal biotin content was perhaps to have been expected since even actively regenerating liver showed no increase, and the myometrium in the fourth month of pregnancy also had a normal biotin content.

The Transition from Embryonic to Adult Biotin Levels in Various Organs of the Rat

In the preceding pages, reference has been made repeatedly to the close parallelism which seems to exist between the biotin levels of embryonic tissues and tumors. Certain embryo tissues of the rat; *e.g.*, liver and connective tissue, contain much less vitamin H than the same adult tissues, while with other organs the situation is just reversed. One immediately wonders whether or not the transition from embryonic to adult biotin levels is an abrupt one, whether all organs are involved at once, and whether the time

at which this transformation takes place coincides with any known changes in metabolism of the tissues concerned.

Rat fetuses were removed at varying stages of development, the age being estimated from the age-weight tables of Donaldson (4). Sufficient embryo livers could be obtained as early as 5 days before birth, kidney at 3½ days, and other organs at 1½ days. Further samples of these organs were taken for assay at the time of birth and at intervals thereafter in order to follow closely the changes in biotin activity over the period just before and after delivery. These data are summarized in Fig. 2. The rapid shift in the

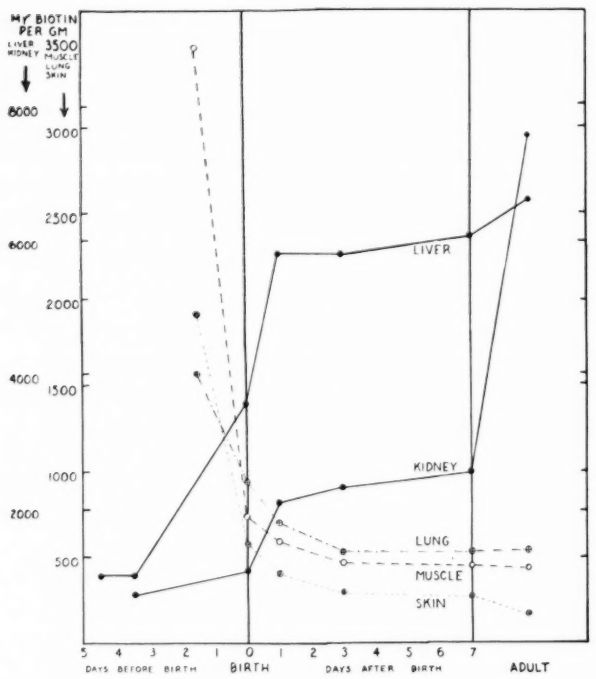

Fig. 2.—The biotin levels of various rat organs before and after birth.

distribution of biotin among the representative organs shown is most striking. Beginning a day or so before birth, those organs characterized by a higher biotin activity in the embryonic stage lose vitamin H very quickly, and by the third post-natal day the normal adult values are reached. At exactly the same time that this precipitous decline in biotin activity is occurring in some organs, liver, which is characteristically low in the embryo as compared with the adult, shows a sudden rise, and also within 3 days after birth attains normal adult levels. As indicated in Fig. 2, the situation in the case of kidney is somewhat more complex but the same general trends are evident.

As to the meaning of these drastic readjustments of biotin value in the various organs, one can at present only hazard a guess. Comparisons made between the biotin contents of the organs of a 4½-month human fetus and the organs of normal adult persons autopsied after accidental death, present the same picture with only minor modifications. These changes in vitamin H activity might well reflect a fundamental alteration in cellular metabolism occurring at the time of transition from intra-uterine to extra-uterine life. Particularly interesting in this respect is the observation of Norris, Blanchard, and Povolny (10) and of Burk (1) that the high anaerobic glycolysis of embryo liver falls to normal adult values very shortly after birth. This is correlated by them with the decrease in hematopoietic elements in the liver which takes place at this time. Since malignant change is said by some investigators to be accompanied by increased glycolysis, both aerobic and anaerobic (1), and at the same time the biotin levels of the tumors shift back toward those of the corresponding embryo tissues, a close relationship between biotin activity and the unusual carbohydrate metabolism of embryo and tumor appears to be an interesting possibility.

Growth of Transplanted Tumors in Biotin-Depleted Mice

Various attempts were made either to raise or lower the biotin contents of the tissues of experimental animals with the idea of observing the course of transplanted tumors under such altered conditions. It soon became apparent that mice are capable of tolerating enormous doses of biotin extract without any change in the free or total biotin content of the tissues. As illustrated in Table I, mice which had received 200 rat units of SMA vitamin H concentrate per day (0.2 ml. intravenous, 0.2 ml. intraperitoneal), did not show any increase in free biotin whatever but even a slight drop below normal in some cases. Total biotin values also remained constant, the only notable exception being the kidney, where they reached more than three times the normal. Since such results were obtained by giving maximal doses of biotin concentrate, attempts

Table I: Biotin Content * of Organs of Mice Treated as Shown

Organ	Stock diet		Injections of biotin concentrate		Egg white diet		Per cent depletion
	Total	Free	Total	Free	Total	Free†	
Skin	618	78	633	56	76	< 5	88
Muscle	905	65	1010	47	102	< 5	89
Lung	1681	123	1420	75	230	22	87
Liver	3120	106	5330	65	580	12	82
Spleen	585	...	510	..	114	< 5	81
Kidney	3590	128	13,330	100	1075	9	70
Brain	2210	88	2000	54	845	25	63

* mγ biotin per gm. of tissue.
† Free values in group on egg white diet obtained from a group of deficient animals other than those used in total biotin assays.

to raise the biotin content of the tissues had to be discontinued, although it appears possible that if pure biotin were available sufficient could be administered without toxic effects to accomplish this end.

The withdrawal of biotin from the tissues of mice proved to be much simpler. Feeding a diet rich in the biotin inhibitor, avidin (5), produced early signs of deficiency after 6 weeks, severe signs after 12 weeks, and death usually at about the 16th week. For the last few weeks of life all animals appeared cachectic, most of the fur was lost, and the skin was thin, inflamed, and in places scaly. Most of the animals were blind, and many had developed a peculiar spastic gait. Others seemed to be in constant pain, as though the cutaneous sensory nerves were irritated, because they continually licked themselves. Often the lightest touch with the tip of a pencil was sufficient to induce convulsive seizures. The mice seemed to continue in about the same condition for the last few weeks of life, and then died suddenly. Histologically the internal organs showed nothing remarkable; studies on the central nervous system are in progress.

Data given in Table I indicate the extent to which the various tissues were depleted of biotin at the time of death. All the organs assayed except kidney and brain were found to have lost 80 to 90 per cent of their biotin activity. The retention of biotin by kidney and brain is emphasized by other figures obtained at an earlier stage of biotin depletion, at a time when the other organs had already lost approximately 65 per cent of their normal vitamin supply, while kidney and brain had not even begun to be drawn upon and showed normal values.

As to the fate of tumors transplanted into biotin-deficient hosts, two examples will serve to illustrate. When it was estimated that a very deficient lot of animals had approximately 2 to 3 weeks to live, fragments of the rapidly growing sarcomas 37 or 180 were introduced subcutaneously into these and into normal control animals on the standard laboratory ration. These tumors, it will be recalled, are of the type which are biotin-poor in contrast to the corresponding normal tissue. Contrary to expectation all transplants grew rapidly, producing large healthy growths even though the mice during this time were in an extreme stage of biotin depletion. After 17 days the average weight of 8 sarcomas 37 from deficient animals was 0.26 gm., the average of 11 control tumors 0.36. In another group the average weight of 9 sarcomas 180 was 0.99 gm. after 10 days' growth in biotin-deficient mice in comparison with 1.06 gm. for a corresponding set of controls.

It appears therefore, that these two sarcomas, the growth of which is essentially unimpaired by extreme biotin deficiency, differ fundamentally from the normal tissues of the host, which cannot function in the absence of an adequate supply of biotin. It was at first thought that a flourishing neoplasm might rob the host of its already depleted biotin store, but on analysis it was found that the tumors contained only the same small fraction of their normal vitamin H contents as the muscle, lung, and liver of the animal in which they were growing—in this case 20 to 24 per cent.

Further evidence that these two tumors possess the power of carrying on their normal metabolic processes unimpaired by lack of a biotin supply may perhaps be furnished by *in vitro* studies. With the addition of avidin to a protein-free culture medium, in excess of the amount required to bind all of the available biotin (as demonstrated by inability of the biotin-requiring test organism, *Rhizobium,* to grow in it), the cells of sarcomas 37 and 180 continued to multiply actively. It is to be emphasized, however, that this investigation, which later will be made the subject of a separate paper, is mentioned only in passing. The growth of normal tissues *in vitro* in the absence of biotin has not yet been examined, as of course it must be before any significance can be attached to the preliminary observation just cited.

DISCUSSION

The possibility that regulatory substances present in cells in only catalytic amounts might be concerned in the malignant process led to this investigation. Biotin, or vitamin H, the most physiologically active vitamin known, was selected because it has been thought to be essential in the energy-yielding reactions of every normal cell.

Results have shown that if carefully selected malignant tissues be compared with their normal counterparts, striking differences in the biotin levels are found. For the most part, the tumors contained only a fraction of that amount of biotin present in the normal controls. A few types, however, were definitely higher in the vitamin. It is interesting to compare these findings with other recent investigations and to speculate upon the possible interrelationships. For example, the biotin level of liver carcinoma was found to be far below that of normal or vigorously regenerating liver, and Burk (1) has found the respiration of such tumors also to be deficient, while regenerating liver remained unchanged. Greenstein and others (6, 7) reported that the arginase and thymonucleodepolymerase activity in such tumors was reduced while simple hyperplastic liver did not differ from the normal. In a recent paper (8) these investigators report similar results concerning the xanthine dehydrogenase, catalase, and amylase contents of hepatic tissues. Furthermore, they too observed a striking parallelism

between the relative activity of fetal to adult liver tissue and that of hepatic tumor to normal liver.

The classic respiration studies of Warburg (11) and the more recent investigations of Burk and his associates (1, 2) have shown that embryo possesses certain metabolic features in common with tumors. For this reason, the biotin level of embryo tissues corresponding to the neoplasms selected was determined whenever possible, and here again, as with the tumors, it was found that all differed widely from the normal adult values. Furthermore, the deviation of both tumor and embryo was closely parallel. In other words, where the value for an embryonic organ was higher than that for its adult counterpart, the content of the tumor was high in comparison with the adult mother tissues; and where the value for an embryonic organ was lower than that for the corresponding adult structure, the tumor contained less biotin than the adult organ.

A further link between the respiratory studies of Burk (1) and the present observations is to be found in a comparison of changes occurring during the transition from embryo to adult. Immediately after birth the biotin content of the various organs of the rat undergoes rapid change and reaches the normal adult values within 3 days. Likewise, the embryo type of glucose disassimilation shifts to that characteristic of the adult very quickly after birth.

It also appears of significance that certain transplantable sarcomas were found to be capable of abundant growth seemingly independent of a supply of biotin, even though they apparently have no power to synthesize the substance. During the last week of life in animals dying from biotin deficiency these tumors were quite unimpaired by lack of this vitamin although in its relative absence the normal host tissues were unable to live.

Hitherto, the various organisms capable of growth in the absence of biotin have been shown to possess the power of synthesizing this substance themselves (9). So far as is known biotin is necessary to all living tissues and if, as is the case with certain bacteria, yeasts, fungi, and mammals, the ability to synthesize biotin has been lost, they become dependent upon an outside source for their existence. However, the two sarcomas mentioned appear to fall in a different category, for it seems that they neither synthesize this vitamin, nor depend upon an extracellular source of supply for their well-being. Perhaps this is in some way connected with the fact that even in a normal animal these tumors are biotin-poor.

The most interesting fact to emerge from the whole investigation, and perhaps the most significant, is this demonstration that the cells of the two sarcomas so far examined, unlike any other cells yet known, are almost, if not quite, independent of biotin. Should this prove true of other tumors in various species it would suggest that biotin is of little or no moment in the life of neoplastic tissues, possibly because of their altered carbohydrate metabolism, and that any shifts observed in the level of this vitamin are a consequence, and not a cause, of malignancy. Nor is other evidence lacking to support such a suggestion. For example, the biotin content of the Shope papilloma, already high, does not rise with the advent of the carcinomatous transformation. Furthermore, young transplanted hepatic carcinomas of the mouse have a biotin level similar to that of normal liver from this species, and not until they have approximately doubled in size do they show the low value that has been found in butter yellow carcinoma of the rat's liver. Yet at a time when they weigh half a gram they are just as truly carcinomas as they are when they have come to weigh one gram.

SUMMARY

1. With but one exception all malignant tumors studied, whether of animal or human origin, differed widely in their biotin content from the corresponding normal tissues.

2. The biotin levels of embryo tissues also diverged widely from those of normal adult tissues, the major shift from embryonic to adult biotin values occurring in the rat within 3 days after birth.

3. In all cases, the biotin content of both tumor and embryo tissues deviated in the same direction from the corresponding normal adult levels.

4. Attempts to raise the general biotin content of animals by injection of biotin concentrate were unsatisfactory because of rapid excretion of the excess vitamin.

5. Both tumors and normal tissues could be greatly depleted of biotin by maintaining the animals on a ration containing avidin, and although the animals suffered severe deficiency symptoms as a result, the growth of certain transplanted tumors was unimpaired. Thus, while biotin is known to be an essential factor in the metabolism of normal mammalian tissues, these tumors, at least, are capable of maintaining their usual activity in its relative absence.

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The Transplantation of an Adenocarcinoma of the Preputial Gland in Mice of the A Strain

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The rare occurrence of neoplastic conditions in the preputial glands of mice would alone justify a note upon the appearance of an adenocarcinoma of this gland in a mouse of the Strong A strain (4). Coupled with this observation are the facts that 1. the tumor has been successfully grafted subcutaneously into other mice of the same strain, and 2. this rare tumor has originated sporadically; that is, the mouse giving rise to the tumor was apparently incapable of transmitting, by heredity, any tendency to give rise to similar tumors of the preputial gland in mice obtained in direct descent.

Haaland (2) in 1911 reported the occurrence of two tumors of the preputial gland in mice. In one case (mouse No. 297) "histologically the picture is exactly that of the normal preputial gland, so similar that the two pictures are hardly distinguishable. The differentiation into fatty masses in the tumour is the same as that of the normal gland. In spite of thus being without distinctive histological differences from normal gland, it exhibits typical malignant growth. . . . . This tumour was transplanted successfully into normal mice, and has been propagated during 11 months through six generations, although with considerable difficulty as compared with other tumours. The percentage of successful inoculations has always been very low, the tumours appear late, grow slowly, and nearly all tumours show a tendency to disappear spontaneously after 3-6 weeks growth. In later generations the tumours show a tendency to grow for a longer time, 8-10 weeks, before absorption sets in." A second tumor (mouse No. 466) gave rise to a very similar tumor, which behaved like the first one in that the growth of the grafted tumor "was only temporary, followed by spontaneous absorption." A certain degree of squamous metaplasia ("kerato-hyalin granules in places and formation of squames") was observed.

MATERIALS, METHODS, AND RESULTS

Male mouse No. S157331 of the F_{57} generation was born August 14, 1939. On June 12, 1940, a swelling about 1.5 cm. on the right side of the penis was observed. As this is often the site of abscess formation in mice, the mass was punctured by a small scissors and mildly queezed. Since no exudate was forthcoming the animal was killed and subjected to autopsy. The solid tumor apparently affected only the right preputial gland. It measured $1 \times 1 \times 1.5$ cm. and appeared to affect the entire gland (from near the base of the penis to its distal end). It pressed upon but apparently did not involve the left preputial gland. A photograph of the intact tumor is given in Fig. 1.

The histological appearance of the tumor is shown in Figs. 4 and 5. Thus it appears to be more cellular than the two preputial tumors described by Haaland. Sections of a preputial gland of a normal mouse (Fig. 2) and of the normal left preputial gland of mouse No. S157331 (Fig. 3) are given for comparison.

The tumor has been successfully grafted into 56 male mice of the A strain through 5 transfer generations (100 per cent takes). In contradistinction to the observation of Haaland that the two tumors described by him showed spontaneous absorption following a period of temporary growth, this new tumor continued to grow progressively until the death of the animal. The growth is expansive, not infiltrative, and has so far never shown any tendency to metastasize. Usually, however, the central area becomes hollowed out or filled with a soft milky liquid material. When approximately 1.5 cm. in diameter the tumor usually ulcerates to the surface, thus discharging the centrally located caseous material. From that point onward the tumor grows expansively from around the periphery of the denuded mass. Photomicrographs of the grafted tumor are given in the T_1, T_2, and T_3 transfer generations in Figs. 6, 7, and 8 respectively.

Male mouse No. S157331 had been mated to its own sister, mouse No. S157330 prior to the discovery of the adenocarcinoma of the preputial gland. From this mating were obtained 4 females and 1 male. Twenty-four males in direct descent from male mouse No. S157331 have so far lived beyond the age of 10 months (10 to 18 months) at which time the original preputial tumor was obtained, without a single occurrence of another tumor of similar origin. It is reasonably clear, therefore, that the tendency to give rise to preputial tumors in the Strong A strain is not determined primarily by heredity; that is, that the present tumor must have originated sporadically.

Backcrossing was not possible because the mouse bearing the original tumor was dead when the adenocarcinoma was discovered.

* This investigation was aided by grants from The Anna Fuller Fund and The Jane Coffin Childs Memorial Fund for Medical Research.

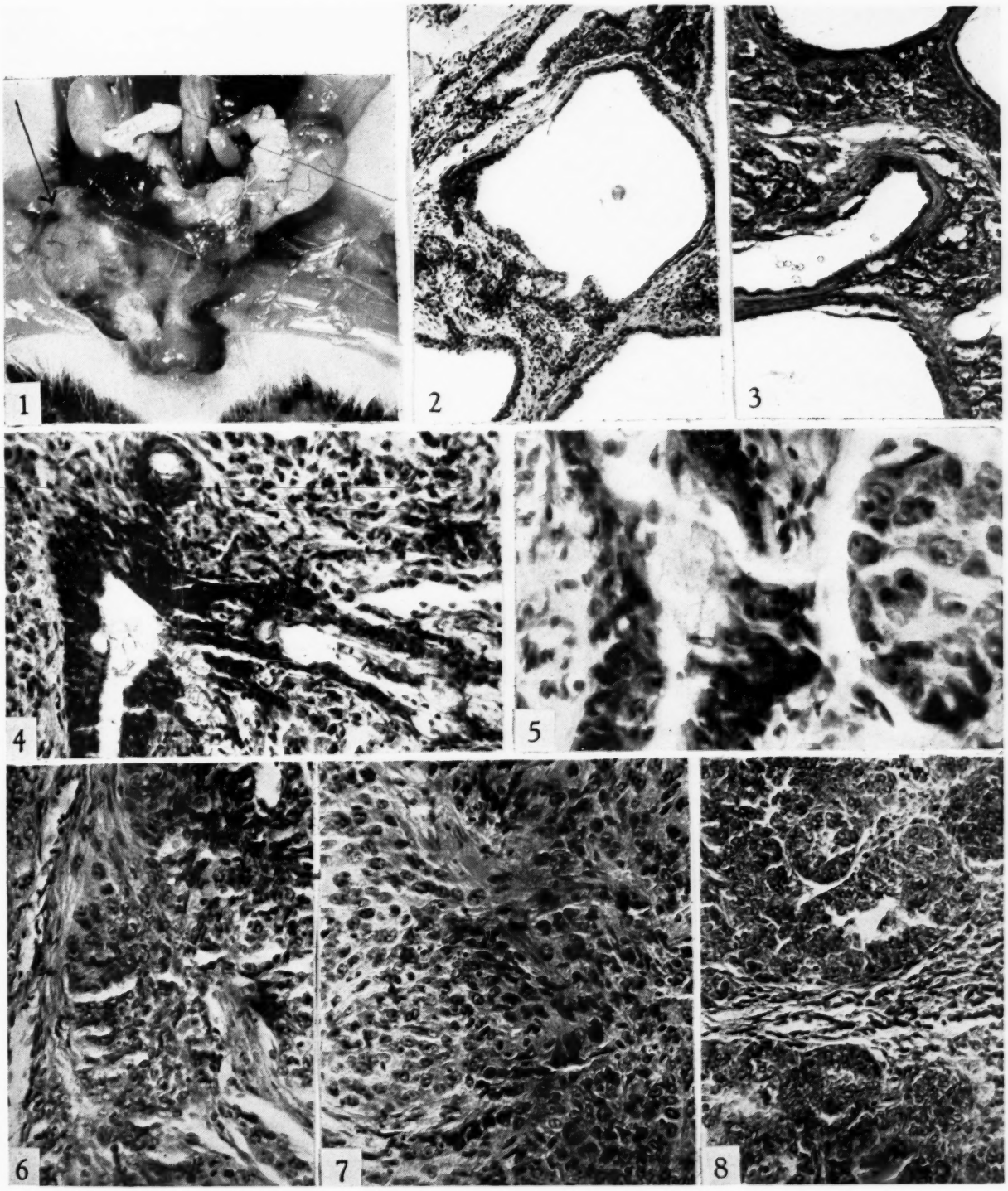

FIG. 1.—Photograph of portion of mouse No. S157331 showing a spontaneous adenocarcinoma of the right preputial gland. Approx. natural size. Arrow points to the tumor.

FIG. 2.—Photomicrograph of section of the preputial gland of a normal mouse of the A strain. Mag. × 100.

FIG. 3.—Photomicrograph of section of the normal left preputial gland of mouse No. S157331. Mag. × 100.

FIG. 4.—Section of the spontaneous adenocarcinoma of the preputial gland of mouse No. S157331. Mag. × 230.

FIG. 5.—Area from the same tumor. Mag. × 475.

FIGS. 6, 7, and 8.—Sections of the transplanted adenocarcinoma of the preputial gland in T_1, T_2, and T_3 transplant generations respectively. Mag. × 230.

DISCUSSION

In 1928 (3) data were published which indicated two conclusions bearing upon the present problem (1). Three tumors derived from connective tissue elements (a) a melanoma, (b) a small round cell sarcoma, and (c) a lymphoblastoma occurred sporadically, and (2) the mice that had given rise to these tumors were incapable of transmitting any tendency to give rise to similar tumors to their descendants. More recently it was shown that a similar phenomenon of the noninheritance of a tendency to give rise to spontaneous squamous carcinoma of the anus occurred in a mouse of the F strain (5). Recently Bittner (1) has recognized the occurrence of this noninherited class of neoplasia as applied to certain cases of carcinoma of the mammary gland. He has expressed the opinion that two fundamental types of carcinoma of the mammary gland may occur in mice. These are (a) that type which is initiated by some influence from the mother's milk acting in conjunction with other conditions and (b) another type which occurs sporadically or *de novo* and apparently is not transmitted to mice of their direct descent. Thus it seems clear that all the above-mentioned tumors are initiated by processes other than those brought about directly by "gene" action from the germ plasm. It must be borne in mind, however, that gene action may be involved indirectly in two ways. 1. There is a possibility that a process of somatic mutation may explain the appearance of sporadic tumors, and 2. the activity of genes may be involved in the building up of a peculiar physiological state, which may permit or culminate in a secondary change necessary for neoplasia. If this physiological state is the result of inherited gene action and the secondary change is, for example, purely somatic gene action or some nongenetic change, then one may or may not expect the tendency to give rise to these sporadic tumors to be exhibited.

Evidence is increasing that the "genetic complex" involved in cancer susceptibility is manifesting itself as the aging process in either local parts or in the organism as a whole. Such a process is of necessity a complex one and the final analysis is not yet at hand.

The adenocarcinoma of the preputial gland of the mouse of the A strain, described in this paper, must be added to the above list in that the tendency to give rise to this tumor was not transmitted by heredity to the small number of male mice available in direct descent.

The successful transplantation of this tumor into all male mice of the A strain is further proof that inbreeding over a period of years has produced mice of more uniform response—an advance over the mice available in Haaland's day.

SUMMARY AND CONCLUSIONS

1. An adenocarcinoma of the preputial gland in mice has occurred sporadically.

2. A tendency to transmit susceptibility to the same type of tumor in mice in direct descent could not be demonstrated.

3. The present observation that a tendency to develop sporadically a rare tumor is not inherited thus emphasizes a class of neoplasia including 1. melanoma, 2. lymphoblastoma, 3. small round cell sarcoma, 4. squamous carcinoma of the anus, 5. certain cases of carcinoma of the mammary gland, and 6. adenocarcinoma of the preputial gland.

4. The rare type of spontaneous tumor occurring in a mouse of a highly inbred strain will grow subcutaneously in all mice inoculated even though the tendency to give rise to the spontaneous tumor is not inherited, thus further emphasizing the idea that the mechanism involved in the transplantation of neoplastic tissue is unquestionably independent of the mechanism that determines the origin and nature of the spontaneous tumor in mice.

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Fluorescent Porphyrins in Harderian Glands and Susceptibility to Spontaneous Mammary Carcinoma in Mice*

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During the quantitative determination of the xanthine oxidase (dehydrogenase) activity in livers of mice of mammary cancer-susceptible and cancer-resistant strains (11), the unused parts of the mice were dissected and examined under near-ultraviolet light. One motive for doing this was related to the fact that one of us had been collaborating with another group, attempting to trace the glandular source of the red fluorescent exudate which had accumulated on the nose and whiskers of pantothenic acid-deficient rats (22). During this work, the very brilliant red fluorescence of the Harderian glands of rats was observed. Mainly out of curiosity, Harderian and lacrimal glands and contents of orbital cavities of all available animals were examined in near-ultraviolet light. No red fluorescence was observed in the contents of the orbital cavities of monkeys, dogs, cats, squirrels, rabbits, guinea pigs, or an opossum.

Mice of the JK cancer-resistant strain and the C3H cancer-susceptible strain were available from the xanthine oxidase (dehydrogenase) determinations. When the Harderian glands from the mice of these two strains were dissected in near-ultraviolet light or so-called "black light" (General Electric Type B-H4 lamp)[1] a remarkable observation was made. The Harderian glands of the C3H mammary cancer-susceptible mouse exhibited a red fluorescence, but the glands of the JK mammary cancer-resistant mouse fluoresced pale white. These procedures and observations were repeated 3 more times with exactly the same results before it was decided to investigate the phenomenon more carefully.

In rats, the red fluorescence of the Harderian glands had been shown to be due to the presence of a protoporphyrin (7, 22). In the case of the mouse Harderian gland, the red fluorescent material is in all probability also a porphyrin. It was soluble in acetic acid and when this was neutralized, it could be extracted with ether. It was removed from the ether with 8 per cent hydrochloric acid but not with 0.5 per cent. It was also soluble in chloroform. The close phylogenetic relationship between rats and mice, the shade of red fluorescence of the material, and the solubility characteristics made it reasonable to assume that the red fluorescence was due to a porphyrin. It is probably mostly protoporphyrin, but until this has been analyzed more thoroughly it is unsafe to be more specific.

A direct relationship between the red fluorescence of the Harderian gland (a lacrimal gland) and cancer susceptibility was regarded as highly improbable. The striking difference in the red porphyrin fluorescence of this gland in mice of cancer-susceptible and cancer-resistant strains was taken to indicate a difference in porphyrin metabolism in mice of the two strains. That such a difference in porphyrin metabolism might be directly or indirectly related to cancer susceptibility is not regarded as improbable for the reasons that will be apparent from a brief consideration of porphyrins.

Porphyrins occur in practically all forms of life (both plants and animals) that exhibit normal and atypical growth. This is because chlorophyll and hemoglobin and similar large molecules contain porphyrins as active constituents. Some of the most important respiratory enzymes such as cytochrome *c*, catalase, the Pasteur enzyme, and peroxidase also contain porphyrins as active parts of the molecule (19, 27, 28, 40, 41). The fundamental nature of the relationship of all of these substances to cellular physiology, especially to oxidations and reductions, cannot be questioned.

From these initial observations and considerations, it appeared that there might be some direct or indirect relationship between susceptibility to spontaneous mammary carcinoma and porphyrin metabolism, or factors regulating this. The first step which has been taken to investigate this possibility was to see if this observation could be extended to mice of other strains in which the degree of susceptibility to spontaneous mammary cancer was known to vary quantitatively from that found in C3H female mice. As mice from strain after strain were examined, the observed intensity of the red fluorescence of the Harderian gland paralleled the previously established degree of susceptibility to cancer. The large number of animals examined, the number of strains analyzed, and the close parallelism observed, indicate that this association was probably not due to coincidence. The preliminary report described the observations on a limited number of C3H and JK mice (34). The extension of the examinations and analysis to include mice in other strains will be described here.

MATERIALS AND METHODS

The mice used in this investigation were from the colony of L. C. Strong. It was desirable for at least two reasons to make a survey of as many strains of

* This investigation was aided by grants from The Anna Fuller Fund and The Jane Coffin Childs Memorial Fund for Medical Research.

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[1] Reflector of Alzak Aluminum; radiation range 4000 to 3100 A.; max. at 3650 A.

mice as possible: 1. in order to ascertain whether or not there was a significant degree of variability in the fluorescence of the exposed Harderian glands of mice of the different strains, and 2. to see if this variability paralleled the variation in susceptibility to cancer. Mice of 13 inbred strains and of 3 hybrid lines were used. These are listed in Table I.

It should be noted that these 13 inbred strains can be classified into 3 groups depending upon the degree of susceptibility to spontaneous cancer of the mammary gland of the females. High susceptibility to spontaneous breast cancer is found in female mice of the C3H and A strains; intermediate degrees of susceptibility in female mice of the C, C12I, CHI, CBAN, and CBA strains; low susceptibility or complete resistance in female mice of the JK, I, F, C57, N, and L strains. F mice, however, give rise to spontaneous myelogenous and lymphatic leukemia as indicated by the work of Kirschbaum and Strong (21). Of the hybrid mice used, not enough is known of their tendency to develop spontaneous tumors of breast tissue to warrant classification into one of these 3 classes. The incidence of spontaneous mammary cancer in NH and NHO mice is, up to the present time, nonexistent. In addition to this resistance to spontaneous tumors, the NHO mice have been produced by selection toward a high resistance to induced tumors by methylcholanthrene. The FC mice have, so far, never developed spontaneous tumors of any kind.

TABLE I: LIST OF STRAINS OF MICE USED IN EXPERIMENTS

Strain *	Inbred generations	Number of mice used
C3H	56	275
A	74	48
JK	40	68
I	34	37
C12I	48	52
F	36	5
N	36	26
CHI	50	12
CBA	52	5
C	51	11
CBAN	19	6
L	37	4
C57	16	52
NH (unselected)	13	80
NHO (selected)	10	25
FC	7	33
BC to L	..	14
Total		753

* All stocks, except C57, were originated and maintained by L. C. Strong. The C57 was obtained from Dr. H. J. Bagg of New York City and continued in this laboratory for the past 3 years. The unselected NH strain was developed without selection from a cross between mice of the CBAN and JK strains. The selected NHO strain was established by selection toward resistance to tumors induced by methylcholanthrene. The FC strain was obtained by a cross between mice of the F and C57 strains. The BC to L mice were produced by a backcross to the L strain mice from an original cross between L and JK mice.

All mice were maintained on a diet of Nurishmix and water ad libitum and given fresh, washed lettuce twice a week. They were killed either by pinching the spinal cord in the cervical region between the thumb and forefinger or by means of illuminating gas. The skin over the eye was cut away by fine scissors and the eyeball extracted by pulling it out with forceps. The Harderian gland was then grasped with a pair of fine forceps and pulled away from its loose attachments in the orbital cavity. It was then placed on blotting paper where all traces of blood and connective tissue were removed. The glands were then stretched on a clean microscopic slide and pressed down slightly with forceps. All glands were then examined under near-ultraviolet light (General Electric Type B-H4 "black" lamp)[1] within a few minutes after killing.

A method for quantitative determination of the intensity of the red fluorescence of a single gland has not as yet been devised. In this investigation, semiquantitative estimates of the degree of red fluorescence of Harderian glands were made by visual inspection and comparison. The current was turned on several minutes before the examination and estimation in order to permit the lamp to reach its maximal intensity. In nearly all cases, however, mice from many stocks were examined at the same time, the Harderian glands of 25 to 30 mice being placed on the same microscope slide. By these procedures, the personal element and the possible fluctuations in the source of near-ultraviolet light were somewhat controlled.

In order to evaluate the different degrees of fluorescence encountered, an arbitrary division into 6 classes was set up (one more than was used in the preliminary report). These were 0, +, ++, +++, ++++, +++++. The 0 class was used to designate the absence of all traces of red fluorescence. These glands appeared to fluoresce white or light tan in near-ultraviolet light. Occasionally a tiny spot of pink fluorescence would show in one end of the Harderian gland. The maximum degree of fluorescence was designated as +++++; here the red fluorescence was very intense and was comparable to the condition found in normal rat Harderian glands. Glands showing intermediate degrees of fluorescence were classified as +, ++, +++, or ++++, depending upon the estimation of fluorescent intensity.

RESULTS

A great variation in the degree of red fluorescence of the Harderian glands in mice of the various strains was observed. Since the intensity of fluorescence was quite constant in the two glands of the same mouse, it was evident that they were both apparently in the same physiological state at the same time. The only variability encountered in the two glands of the same

mouse was seen occasionally in those mice which approached the 0 class; here one gland would give completely white fluorescence, whereas the other would show a small localized area of very light pink fluorescence. Mice of the same inbred stock and age showed constant degrees of fluorescence. At a given age, however, the females tended to show, on the average, a slightly greater degree of fluorescence than that of the males, but this finding was not always constant. The maximum degree of difference between mice of the different strains was found during early sexual maturity in the C3H (+++++) on one hand and in the old JK, N, and C57 (0) on the other. Harderian glands of mice of the other strains showed intermediate degrees of fluorescence (+, ++, +++, and ++++).

This difference between the strains was not absolute since variations with age occurred. Before the eyes were open at 14 days, there was no red fluorescence evident in the contents of the orbital cavities of any mouse. Shortly after the eyes were open there was a rapid increase in red fluorescence which reached its maximum development at approximately 100 days of life (in JK mice this maximum reading was ++ and in C3H mice, +++++). After that time there was a rapid drop in the red fluorescence of mice of the JK strain which completely disappeared in middle sexual maturity, and this absence of red fluorescence then persisted during the rest of the life span. On the other hand, there was a much slower decline in the intensity of red fluorescence in mice of the C3H strain with advancing age. Only one individual of the C3H strain, at 29 months of life, had no red fluorescence.[2] The changes in fluorescence with age are shown for the JK and C3H mice in Fig. 1.

In contrast to this constancy of fluorescence in individuals of the same age and inbred strains, there was considerable variability in fluorescence in individuals of the same age belonging to the hybrid lines FC, NH, and NHO. That is, when there was a high degree of genetic uniformity between individuals of the same inbred strain and age, uniformity of red fluorescence was seen. When there was marked genetic variability among the individuals (in hybrid generations following an outcross) a diversity of fluorescence was seen among the individuals.

Since a few backcross mice to the L stock (from a cross between JK and L) were available, these were examined under near-ultraviolet light. They all approached the reading of fluorescence determined in mice of the L strain, thus indicating that the presence of porphyrins causing red fluorescence of the Harderian glands may be an inherited dominant characteristic.

As an incidental finding, it was observed that the color of the Harderian glands in mice of the different strains was variable even in visible light due to the relative abundance of a dark pigment. For example, mice of the A strain (with pink eyes) have practically flesh-colored Harderian glands under visible light; mice of the C3H strain (with black eyes) have dark gray Harderian glands under the same light. This "melanin-like" pigmentation, however, is not necessarily correlated with eye color, since mice of the N strain with black eyes have flesh-colored Harderian glands. Whether or not this pigmentation or any other constituent of the Harderian gland has any effect on the presence or absence, abundance or protection of porphyrins is not clear. Hybrid mice of the FC descent (all with black eyes) showed variable degrees of pigmentation under visible light. At least four grades could be detected in litter mates as follows: (a) flesh color, (b) light gray, (c) medium gray, and (d) dark gray. This variability was not seen in mice of the inbred strains, thus indicating that the presence of this pigment may have a genetic basis. As a general rule, females show more of this gray pigmentation than do litter-mate males. Mice with the gene for brown show lighter Harderian glands than mice with the gene for black coat color. There is a decrease in the amount of gray pigmentation as well as red fluorescence with advancing age in both males and females after early sexual maturity.

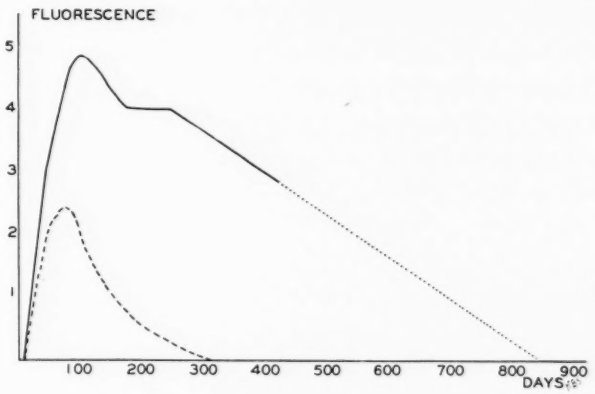

FIG. 1.—The data on the degree of fluorescence obtained by the exposure of Harderian glands to near-ultraviolet light. Age in days is given on the base line; the degree of estimated fluorescence upon the perpendicular. Data for the JK mice are given on the short dash line; those for the C3H mice are presented on the solid line; the projected dotted line for the C3H curve is given to the zero point obtained by a single determination.

One apparent exception to the rule that mice with high susceptibility to spontaneous cancer show maximal red fluorescence of the Harderian gland was seen in mice of the A strain. Instead of showing +++++

[2] From Dr. W. U. Gardner. This mouse was not on Nurishmix diet. This C3H female must also have been somewhat resistant to cancer, otherwise this age would not have been attained.

fluorescence, as might be expected, some of the mice showed the amount characteristic of those mice showing intermediate degrees of susceptibility (++ or +++). The reason for this is unknown. Whether or not the absence of the dark visible pigment (as is observed in A strain mice) permits a rapid decomposition of the red fluorescent porphyrin of that gland has not been determined. The fluorescence in the dried Harderian gland of the C3H mouse persists longer than does the fluorescence in a gland from a mouse with an intermediate reading. This may be due to an originally greater amount of porphyrin rather than to the possibility that the substance is protected from deterioration by special conditions existing in that gland.

In contrast to the findings obtained many times on mice of other strains it was noted that the red fluorescence of the Harderian gland in mice of the A strain seemed to increase temporarily in intensity after the gland had been placed on a microscope slide for a short time. Therefore, this lower degree of red fluorescence in mice of the A strain may not be real, but may be due to some peculiar condition within the gland that masks or prevents the full manifestation of fluorescence. The Harderian glands of all mice lose their red fluorescence when kept on a microscope slide at room temperature. The capacity to give the characteristic fluorescence may be retained at least for several weeks by keeping the glands frozen.

DISCUSSION

In discussing the close parallelism which was found to exist between the degree of cancer susceptibility and the variations in porphyrin metabolism as indicated by different degrees of the intensity of red fluorescence of Harderian glands, two main possibilities must be considered. One is that this observed parallelism is due to mere coincidental association of these two characters which may be otherwise unrelated to each other. Such associations are sometimes observed in genetic studies. The second possibility is that some fundamental direct or indirect interrelationship may exist between these two characteristics.

The apparently paradoxical situation in the case of rats would seem to lend support to the first possibility. The Harderian gland of the rat exhibits an intense red fluorescence, yet rats rarely develop spontaneous mammary carcinoma. This fact could be regarded as evidence in favor of the hypothesis that cancer susceptibility and factors influencing porphyrin production and metabolism are independent and unrelated and that their close association in mice is merely due to a chance coincidence. However, if we assume, on the basis of the red fluorescence of the Harderian glands, that certain closely related factors which help regulate both porphyrin metabolism and cancer susceptibility are present in both rats and cancer-susceptible mice, it does not follow that there should be a high incidence of spontaneous mammary carcinoma in both. Some additional interrelated factors which influence susceptibility to cancer may be operating in the mouse and not in the rat. Likewise, other factors regulating the presence or potency of carcinogenic stimuli may be present in the mouse and not in the rat. On logical grounds, therefore, the fact that the rat has a brilliant red fluorescent Harderian gland and yet low incidence of or resistance to spontaneous mammary carcinoma cannot be used as an argument to disprove the hypothesis that factors that regulate porphyrin metabolism are also concerned in the determination of cancer susceptibility.

The other possibility; namely, that a fundamental relationship exists between the factors regulating porphyrin metabolism and the factors determining susceptibility to cancer, is supported by the observed close parallelism and by some other meager evidence. The facts which indicate a possible direct relationship will be considered first and the more conservative concept of an indirect relationship will be mentioned last.

At first it was thought that excess porphyrins might have a direct effect on the susceptibility to a given carcinogenic stimulus. The high concentration of porphyrin in the Harderian glands of cancer-susceptible mice which is responsible for the red fluorescence, is not necessarily an indication of the porphyrin concentrations of other organs and tissues. If the Harderian gland does not elaborate the porphyrin but extracts it from the blood, it is conceivable that the continuous secretion of porphyrin by this gland would decrease rather than increase the concentration of porphyrins elsewhere. On the other hand, if the Harderian gland does elaborate the porphyrin, which is responsible for the more persistent and intense red fluorescence observed in the cancer-susceptible mouse, then it is highly probable that this gland is responsible for a generally higher porphyrin level in other organs and tissues.

This statement is made on the basis of the following observations in rats: If rats are deprived of water, the red fluorescent secretions of the Harderian glands accumulate on the noses of the rats (9). When the rats are then allowed to drink water, they soon clean the red fluorescent material off the nose and fur. Such rats, when killed 4 hours after the cleaning process, exhibit a strong red fluorescent band in the intestine due to the fluorescence of the unusually large amount of material cleaned off by washing and licking. The feces of rats are red fluorescent, due to presence of porphyrins (25). When the Harderian

glands are removed from rats (10), the feces lose the red fluorescence. These facts indicate that the Harderian gland in the normal rat, while it is presumably an excretory gland, secretes a porphyrin that probably finds its way back into the blood stream by way of the nasolacrimal duct, the nose, the nasopharynx, and the alimentary tract. If only a part of the porphyrin be absorbed from the alimentary tract, it would thus be possible for a porphyrin-elaborating Harderian gland to increase the concentration of porphyrins in other organs and tissues. The influence which the Harderian gland has on the concentration of porphyrins elsewhere depends on whether or not this substance is elaborated in the Harderian gland and this is not known.

In addition to uncertainty as to whether the secretion of porphyrins by the Harderian gland increases or decreases the concentration of porphyrins in other organs or tissues, there is little or no evidence to indicate that variations in porphyrin concentration would influence either cancer or susceptibility to cancer. Some fragmentary but suggestive data that may have a bearing on this question may be mentioned. The fact that porphyrins are present in bone marrow as a necessary step in the synthesis of hemoglobin may be relevant. Throughout life the bone marrow is one of the most active tissues as regards cellular proliferation. According to Borst and Königsdörffer (5) the megaloblasts and erythroblasts contain a relatively large amount of protoporphyrin. Porphyrins were demonstrated by fluorescence studies in the central necrotic inoculation center in induced rat sarcoma (24). This was thought, however, to be of bacterial origin. More recently, Thomas (37) has described the fluorescence of the tumor masses in cases of chloroma. As indicated by the name, these masses appear green in visible light but they are red fluorescent in near-ultraviolet light. Thomas identified the red fluorescent pigment in these tumor masses as protoporphyrin (37). In addition he was able to extract protoporphyrin from the red fluorescent centers of lymph nodes in two cases of myelogenous leukemia. This red fluorescent pigment was also extracted and proved to be protoporphyrin.

Estrogenic effects are also claimed for porphyrins by Rodewald (26) who reported that a series of several estrus stages may be induced in ovariectomized mice by a single series of injections of porphyrin. This work, however, lacks confirmation. The same author reported that injections of porphyrins stimulate the hypophysis to secrete larger amounts of melanophore-dispersing and gonadotropic hormones. In the serum of injected animals, a substance which inactivated the melanophore-dispersing hormone was demonstrated which was thought to be identical with the one found in the serum of patients with cancer (20). Dobriner and Rhoads (8) treated rabbits with 1,2,5,6-dibenzanthracene and observed increased porphyrin output in the urine. Except for the mention of the work on dibenzanthracene and porphyrin, recent reviews (8, 39) do not refer to any relationship between high porphyrin levels and cancer.

The photosensitizing action of the porphyrins, demonstrated by Hausmann (18), has been amply confirmed by numerous experiments. One of the most impressive is the experiment of Meyer-Betz (23) who injected himself with 200 mgm. of hematoporphyrin. He developed a severe edema of the skin in areas exposed to light. This photosensitivity lasted several months. In this connection, it would be of interest to determine whether the ultraviolet radiation technic used to induce cancer in albino mice would be as effective in mice with nonfluorescent Harderian glands (4).

The first working hypothesis that developed from the present data and these considerations was that excess porphyrins might influence cancer susceptibility by sensitizing cells to such agents as light, or chemicals formed by the action of light, estrogenic, carcinogenic, and perhaps other similar substances. In addition to the work on estrogenic effects of porphyrins which may be interpreted in terms of increased sensitivity to normally occurring chemicals, there are certain isolated facts that were observed in patients excreting abnormally large amounts of porphyrin, that support this view. In one case, hirsutism was observed in a female. In describing cases of acute porphyria, Grünewald (16) described a virgin who secreted colostrum. Harbitz (17) in discussing another case, described the swelling of the breast of a man during an attack of acute porphyria.

It is, probably, more conservative to postulate a relationship between the factors that regulate porphyrin metabolism and those that determine susceptibility to cancer. To introduce a new concept, such as "the variability in factors regulating porphyrin metabolism" into the already complex sequence of events involved in susceptibility to spontaneous carcinoma of the mammary gland is, perhaps, a hazardous procedure. Especially is this true when so little is definitely known about the factors which regulate the metabolism and effects of porphyrins within the body. That variations in the regulation of its production might affect hemoglobin and many of the important respiratory enzymes such as catalase, cytochrome *c*, and the Pasteur enzyme may be highly significant. But whether these enzymes are definitely involved in the origin of the cancer cell has not been proved. That there are variations from the normal in the above enzyme systems

after the cancer cell has been established may be the resultant and not the cause of the cancer cell.

But quite aside from these fundamental studies on cell metabolism which may eventually provide the key for the interpretation of the origin of neoplastic growth, there are six established concepts which are recognized in one form or another as being involved in the explanation of susceptibility and resistance to spontaneous mammary cancer. These may be briefly classified as 1. the dietary, 2. the physiological use or forced breeding, 3. the milk influence, 4. the hormonal, 5. the chemical, and 6. the genetic.

In reference to these, two questions may be asked: (a) Are the contributions from these six fields sufficient to explain completely the sequence of events involved in cancer susceptibility and resistance? (b) Would variations in porphyrin metabolism aid in the interpretation of cancer susceptibility and resistance?

1. The role of diet in cancer susceptibility and resistance is still too little understood. It is known that the incidence of breast cancer as well as other types from other organs can be significantly influenced by changes in the diet, but these alterations are brought about by the use of commercial feeds or by the introduction into the diet of such complex materials as liver (6), etc. Until the mice are supported on synthetic diets, the role of diet or any constituent of the diet cannot be properly evaluated.

2. The physiological use factor of Bagg (1, 2) is an important influence on the incidence of breast cancer. Certainly, however, the effect of use of any part such as the breast must indeed be very complex and much more must be known of what happens to tissues during the process of physiological activity before the final evaluation of this contribution can be made. It is known that without a certain amount of genetic susceptibility, forced breeding does not necessarily increase the incidence of breast tumors. It is also true that in order to bring mice to a level of forced breeding sufficient to lead to the development of breast cancer, maximal conditions of diet must be used so that the dietary influence may be as effective in increasing the incidence of breast cancer as the physiological effects of forced breeding itself.

3. Bittner's milk influence (3) on breast cancer of mice is, perhaps, as clear cut as any influence since the end result or percentage incidence of breast cancer (due to milk supply alone) is remarkably affected. No one is willing, however, to maintain that this influence, even though it be a virus, is sufficient in itself to explain why a particular mouse develops breast cancer or not. Bittner (3) has definitely indicated that a hormonal and a genetic influence must be present in order to explain the incidence of breast tumors in mice raised by foster nursing.

4. A considerable amount of work has shown that the early stimulation of breast tissue by estrogens brings about breast cancer in male mice, providing the male belongs to a genetic strain in which the females show a high incidence of spontaneous breast tumors. But hormonal response does not completely parallel genetic susceptibility. For example, Gardner (13-15) has indicated that it is easier, by hormone injection, to bring about tumors in CBA male mice than it is in A strain males, whereas the susceptibility to spontaneous breast tumors in females of these strains is just the reverse. The doses necessary to bring about tumors by the injection of estrogen are still excessive over prolonged continuous stimulation, and may be far beyond the range found in the normal physiology of the control animal. However, with the use of chemically pure estrogens in pellet form, the chief difference between experimental and normal conditions lies in the fact of a continuous (not actual) supply of the estrogen in experimental mice rather than in the cyclic as occurs in normal female mice. On the other hand, Twombly (38) has shown that by a combination of foster nursing and hormone injections, male mice belonging to a genetically resistant-to-cancer strain may give rise to breast tumors; that is, this combination of two principles apparently produces the same effect as the chemical influence mentioned below.

5. Recently it was shown that the presence of methylcholanthrene in female mice of the NHO strain being used for breeding will bring about breast cancer in about 10 per cent of the animals. So far this phenomenon has been found only in mice belonging to a genetic strain characterized by a low incidence of spontaneous breast tumors. That is, the presence of the carcinogen replaces, as it were, genetic susceptibility. Thus, it is reasonably certain that the mechanism involved in the origin of a methylcholanthrene-induced breast cancer is not necessarily the same as the one involved in a mouse receiving estrogen alone.

6. That a genetic determiner is involved in susceptibility and resistance to spontaneous breast cancer is commonly accepted. Just what role this determiner plays is not yet clear. Many suggestions have been made, some on theoretical grounds, others on experimental evidence, of how a determiner from the germ plasm could alter or influence an individual in such a way that breast cancer will of necessity eventually develop. Among these suggestions based on experimental evidence are those of Strong (12, 29-36) and his collaborators who have endeavored to bridge the gap between the germ plasm and the disease, cancer, by an analysis of the physiological components

of the individual. These studies have shown 1. that there is a precocious drop of hemoglobin in a mouse known to be genetically susceptible to breast cancer as compared to one of a cancer-resistant strain (12, 35, 36); 2. that the mouse belonging to a susceptible-to-cancer strain is less tolerant to salicylaldehyde than one from a resistant strain (32, 33); 3. that a mouse of a strain showing high susceptibility to spontaneous breast cancer has less xanthine (oxidase) dehydrogenase activity in its liver (11); and 4. that the susceptible-to-cancer mouse also shows more intense red fluorescence of the Harderian gland (thus indicating a porphyrin metabolism of a certain type) than a mouse of a resistant-to-cancer strain (34). These seemingly unrelated phenomena may have something in common. With the exception of xanthine dehydrogenase activity of livers, where the available data do not permit a comparison as yet, they follow the same type of distribution in an individual at different age levels; a low initial reading which increases in amount during early sexual life, remaining uniform in middle sexual life, and then diminishing with advancing age. If this be true, then the genetic determiner in cancer susceptibility (of which the above criteria may be indices) may be merely influencing the aging process of individual tissues or the "individual as a whole." Other physiological functions may also be determined later, which also vary according to this general trend with age.

It is likely, however, that the contributions of the six separate fields of biological research enumerated above and bearing upon the problem of the origin of the cancer cell are not in themselves sufficient to explain the changes involved in the conversion of a normal to a neoplastic state. Some concept such as a sensitizing effect of a chemical may be necessary to correlate some of the diverse concepts.

SUMMARY

The finding is reported that mice of strains with high susceptibility to breast cancer show more intense red porphyrin fluorescence of the Harderian glands than do mice belonging to strains with low susceptibility to cancer. The close parallelism between the degree of red fluorescence of the Harderian gland and the degree of cancer susceptibility in over 750 mice of 16 different strains was regarded as evidence in favor of the following hypothesis: There is a direct or indirect relationship between porphyrin metabolism and the factors that determine cancer susceptibility.

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The Reciprocal Infection of Ducks and Chickens with Tumor-Inducing Viruses*

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The present report considers the variations which take place in the causal virus following injection of ducklings with the Rous sarcoma of chickens, and with further variations following the infection of chickens with viruses from the resulting strains of duck tumors. The study of the hemorrhagic lesions often developing in these infections will be taken up in detail in another paper. The great susceptibility of chicks to chicken tumor viruses injected intravenously (4, 5) contrasted with the resistance of adult chickens to similar injections (3, 27) suggested the present investigation. A preliminary note on the subject has been published (6).

In 1912, Murphy and Rous (21) grew chicken tumor I in the membranes of developing duck embryos, but transplantation of the tumors obtained into adult ducks failed. Purdy (26) also failed to infect Khaki Campbell ducklings and ducks by intramuscular injection of large amounts of extracts from Rous tumors and Mill Hill 2 endothelioma, and Des Ligneris (2) had the same experience with adult ducks and geese injected with minced tumor tissue. However, Purdy (26) propagated the Rous sarcoma through 5 serial generations of Khaki Campbell ducklings (and presumably could have continued this propagation indefinitely) by injecting large amounts of minced tumor tissue (0.5 cc.). The age of the host was a very important factor. In 1-day-old ducklings, transplantation was invariably successful, and apparently the same was true for 4-day-old ducks; only half of the ducks 11 to 14 days old were found susceptible, while adult ducks were wholly resistant. The Mill Hill 2 endothelioma, also injected under similar conditions, induced tumors which regressed after one week of growth. Fujinami and Hatano in 1929 (7) reported the successful transplantation of the Fujinami sarcoma through 40 generations of ducks and observed that the tumor grew remarkably well in such hosts. Presumably cells were used for transplantation in these experiments. Gye (14) confirmed these observations on 20 Aylesbury ducklings. He further induced tumors in half-grown ducks by means of a filtrate, but these tumors always regressed. Using the Khaki Campbell breed the same author (13, 14) propagated the Fujinami tumor through 18 serial generations of ducklings by means of filtrates or cell suspensions. Half-grown or older ducks formed tumors in response to injection of cell suspensions or filtrates, but these tumors were always followed by regression. Purdy (24), by using larger doses (2 cc. of minced tumor) than Gye did, found it possible to propagate the tumor through an indefinite number of generations of adult ducks. In some cases, however, the tumors regressed and the host recovered. These ducks were found later to be resistant to reinoculation with the same tumor, but no resistance against the Fujinami tumor was found in ducklings in which the Rous tumor grew for a time and later regressed (25).

Two points concerning these duck tumors should be emphasized. The first is that in no case, even after being carried for 40 generations in ducks, did the virus fail to induce sarcomas in adult chickens. The second is that a generalized neoplastic disease was never observed in ducks; the only evident manifestation of the virus activity was a rapidly growing tumor which killed the host in such a short time that Gye suspected a toxic action (14). These facts indicate that variation of the chicken virus was not observed in any instance.

MATERIALS AND METHODS

Both Rous and Fujinami sarcomas were employed in the present investigation. The Rous sarcoma was used routinely, the Fujinami only occasionally. Both tumors were serially grown in adult Barred Plymouth Rock chickens. Passages were made by injecting a 1:5 suspension of tumor cells in 0.85 per cent saline solution into the breast. No tumor older than 10 days was ever employed. Berkefeld N candles were used for filtration of the tumor extracts. Ducks of the Pekin breed were generally used, other breeds being employed only in a few experiments. They all came from the same farm.[1] The intravenous injections in ducks and chicks were made by way of the jugular vein; subcutaneous and intramuscular injections by way of the breast. Data on the dosage and strength of the tumor preparations injected will be given in the text. Tissue sections were stained with hematoxylin and eosin unless otherwise indicated.

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[1] The cooperation of the Messrs. Robinson of the Carman River Duck Farm, Brookhaven, L. I., in securing these breeds of ducks is acknowledged with pleasure.

In experiments of short duration the birds were kept in individual cages in the animal room. In experiments of long duration chickens and ducks were transferred to a farm where they lived together in a large enclosure of land with running water available to them. If injected shortly after hatching they were kept together in brooders in the laboratory animal room for 6 or 8 weeks, and were then taken to the farm. A chick growing mash was fed ad libitum.

THE INFECTION OF DUCKLINGS BY VIRUS AND CELLS FROM CHICKEN SARCOMAS

In 16 experiments carried out in the course of 20 months, filtered or unfiltered extracts and cell suspensions of Rous and Fujinami tumors were injected intravenously or into the breasts of 236 Pekin ducks of widely differing age. All of these preparations were quite active for chickens. The ducks died of lesions induced by these viruses or of intercurrent infections, or were killed, sometimes as late as 16 months, often 7 months, and rarely 2 months after injection. Details and results of the experiments are recorded in Table I.

TABLE I: AGE SUSCEPTIBILITY OF PEKIN DUCKS TO VIRUS AND CELLS OF CHICKEN SARCOMAS

Tumor material injected	Dose in cc.	Number of ducks	Age of ducks in days	Results at death: Hemorrhagic disease	Sarcomas	Lymphoblastomas	No lesions
Extract, 1:20, in vein (Rous)	1 or less	33	1	..	..	..	33
Extract, 1:20, in vein (Rous)	3–4	50	1	13	3	1	33
Extract, 1:20, in vein (Fujinami)	3	13	1	2	1	..	10
Extract, 1:20, in vein (Rous)	3	18	2–4	..	..	..	18
Extract, 1:20, in vein (Rous)	3–50	9	7–60	..	..	..	9
Extract, 1:20, in vein (Fujinami)	3–10	7	4–20	..	..	..	7
Extract, 1:20, in breast (Rous)	1 or less	24	1	..	..	..	24
Extract, 1:20, in breast (Rous)	3–4	20	1	..	3	..	17 *
Extract, 1:20, in breast (Fujinami)	3	2	1	..	2	..	..
Extract, 1:20, in breast (Rous)	5	5	5	..	..	..	5
Cell suspension, 1:20, in breast (Rous)	0.5	6	1	..	2	..	4
Cell suspension, 1:20, in breast (Rous)	1	7	1	..	5	..	2
Cell suspension, 1:20, in breast (Rous)	2–4	10	1	..	8	..	2
Cell suspension, 1:20, in breast (Rous)	2–4	6	2–4	..	3	..	3
Cell suspension, 1:20, in breast (Rous)	4	2	11	..	..	..	2
Cell suspension, 1:20, in breast (Rous)	1–80	22	30–63	..	..	..	22

* None of these animals had local lesions at the site of injection; 6 ducks developed visceral tumors or hemorrhagic lesions.

One consistent result has been that only 1-day-old ducklings were susceptible to the tumor extracts, and then only when 3 or 4 cc. of the preparation were injected into the vein or breast. Of the 109 animals in this group only 25 (22 per cent) developed noticeable lesions. The samples of extract used, each coming from a different chicken tumor, showed an infectivity for ducks which varied from 0 per cent to 100 per cent. Unfiltered extracts proved to be twice as effective as filtered ones. The period of susceptibility to tumor cells (between 1 and 11 days) was somewhat longer, and the incidence of tumors in the 23 one-day-old ducklings was higher (65 per cent) than when extracts were injected. The data concerning the susceptibility of ducklings to Rous sarcoma cells confirm previous results of Purdy (26).

The results show also that intravenous injections of extracts were more effective than injections into the breast. Thus, two exceptionally potent extracts, which invariably produced lesions if injected intravenously, induced tumors in the breasts of only 5 out of 22 one-day-old ducklings. A less active extract failed to induce tumors in the breasts of 17 ducks but proved to be active in 5 out of 11 ducklings when injected intravenously. Moreover, in 6 of the breast-injected ducklings which did not develop local lesions, autopsies revealed tumors and hemorrhagic lesions in livers and lungs.

Since in the preceding experiment we did not know the exact age in hours of the ducklings shipped to us as 1-day-old birds, we injected 3 cc. of unfiltered tumor extract intravenously into 4 groups of ducklings which ranged from hatching to 5, 10, and 24 hours in age. This was done in 4 separate experiments at the farm supplying our ducks. The results appear in Table II.

TABLE II: SUSCEPTIBILITY OF THE PEKIN DUCK TO THE ROUS AND FUJINAMI VIRUSES DURING THE FIRST 24 HOURS AFTER HATCHING. (3 CC. TUMOR EXTRACT DILUTED 1:20 INJECTED IN VEIN)

Number of ducks	Age	Tumor employed	Results at death: Hemorrhagic disease	Tumors	No lesions
23	Hatching	Rous	3	3	17
9	Hatching	Fujinami	0	1	8
13	5 hours	Rous	0	1	12
7	5 hours	Fujinami	0	0	7
8	10 hours	Rous	1	0	7
9	10 hours	Fujinami	0	0	9
15	24 hours	Rous	1	0	14

It is clear that the greatest susceptibility of the ducks to the tumor viruses was found at hatching and diminished rapidly during the first hours of life.

The lesions induced by the injection into ducklings of extracts from the Rous and Fujinami tumors were either hemorrhagic or neoplastic, and in describing each group a distinction will be made between *immediate* and *late* lesions.

either grossly or microscopically. The animals died from 34 to 101 days after injection. In 8 other cases, recorded as negative in Tables I and II, indications of healed hemorrhagic lesions were found.

Neoplastic lesions.—In 7 ducks, immediate sarcomas developed which killed the animals in 10 to 20 days without obvious generalized lesions. Late tumors developed in 4 apparently healthy ducks which were killed 40 days to 7 months after injection. The tumors induced by the injection of cells fell within the former group. These tumors often killed their

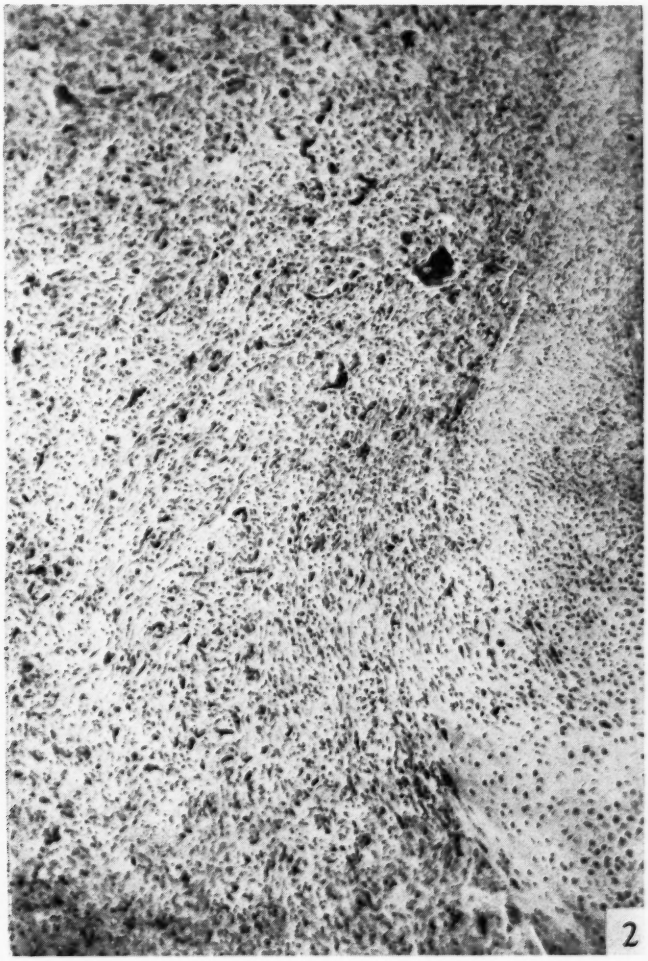


FIG. 1.—Hemorrhagic disease in a duckling injected intravenously 1 day after hatching with extract of Rous tumor, and dying 14 days later. Note minute tumor nodules in the pancreas and neck.

FIG. 2.—Section of periosteal giant cell sarcoma from a bone of the face in a duckling injected intravenously 1 day after hatching with filtrate of Rous tumor, and killed 40 days later. Mag. × 120.

[All photographs were taken by Mr. H. J. Reynolds.]

Hemorrhagic lesions.—An immediate and acute type of hemorrhagic disease was observed in 15 ducklings. The disease was similar to that described in chicks (4), although differing from it in minor features (Fig. 1). Small viscid tumor nodules, mostly around the injected vein, were noticed in 5 cases. Death occurred in from 10 to 25 days after injection. The microscopic study showed that blebs were in most cases without any association with neoplastic tissue. A late type of hemorrhagic disease, never observed in chicks, occurred in 5 ducks. The liver was the only organ affected; it was enlarged, showed pronounced gross and microscopic signs of degeneration, and large organized clots bulged from its surface. No tumor tissue was apparent

hosts from 5 to 20 days after injection usually without generalized lesions, thus confirming a previous finding by Gye (14). In some cases, however, invasion of the liver by direct extension of the growth was observed.

Data concerning the tumors in which transmission was attempted are given in Table III. The few remaining tumors were, with one exception, immediate myxo- or compact spindle cell sarcomas corresponding with those of Table III. The exception was a tumor in a duck of experiment E, which was injected intra-

TABLE III: TRANSMISSIBILITY OF TUMORS INDUCED IN DUCKS BY THE ROUS AND FUJINAMI VIRUSES

Duck number	Experiment	Strain of virus	Strain of ducks	Method of induction	Age of host and tumor in days	Data on duck tumors: Location	Size in cm.	Texture	Histological diagnosis	Transmission: In ducks: Extract, 1:20, 1 cc.	In ducks: Cells, 1:5, 1 cc.	In chickens: Extract, 1:20, 1 cc.	In chickens: Cells, 1:5, 1 cc.	Remarks
1	Z1	Rous	Khaki Campbell	4 cc. cells, 1:20, breast	5	Breast	5.0 × 4.0	Very soft, viscid	Myxosarcoma	−	+	+	+	
2	V2	Rous	Puddle	4 cc. cells, 1:20, breast	7	Breast	5.0 × 4.0	Soft, viscid	Myxosarcoma	..	+	..	..	
3	V3	Fujinami	Puddle	4 cc. cells, 1:20, breast	7	Breast	2.0 × 1.0	Firm, viscid	Myxosarcoma	..	+	..	..	
4	M	Rous	Pekin	4 cc. cells, 1:20, breast	8	Breast	3.0 × 3.0	Soft, viscid	Myxosarcoma	−(filtered)	+	+(filtered)†	+	
5	Z2	Rous	Khaki Campbell	4 cc. cells, 1:20, breast	9	Breast	3.0 × 2.0	Firm, a little viscid	Myxosarcoma	−	+	..	..	Source of strain Z
6	H	Rous	Pekin	4 cc. cells, 1:20, breast	16	Breast	5.0 × 5.0	Soft, viscid	Myxosarcoma	+	+	+†	+	Source of strains HV and HC; duck 4 days old when injected; showed lung metastases at death
7	V4	Rous	Puddle	4 cc. cells, 1:20, breast	18	Breast	3.0 × 3.0	Firm, not viscid	Spindle cell sarcoma	..	+	..	..	Source of strain V4
8	C2	Fujinami	Pekin	3 cc. extract, 1:20, breast	23	Breast	2.0 × 0.4	Resilient, not viscid	Spindle cell sarcoma	−	+	+†	+	
9	T	Rous	Pekin	3 cc. extract, 1:20, breast	30	Breast	7.0 × 5.0	Resilient, not viscid	Spindle cell sarcoma	−(filtered)	..	+(filtered)†	+	
10	S4	Rous	Pekin	3 cc. extract, 1:20, vein	15	Heart	0.8 × 0.4	Firm, not viscid	Spindle cell sarcoma	..	−	..	..	Grafts used for transmission
11	S2	Rous	Pekin	3 cc. extract, 1:20, vein	17	Neck	0.8 × 0.5	Soft, viscid, hemorrhagic	Myxosarcoma	..	−	..	..	Grafts used for transmission
12	S3	Rous	Pekin	3 cc. extract, 1:20, vein	18	Liver	0.4 × 0.3	Soft, viscid	Myxosarcoma	−	..	..	..	
13	C1	Fujinami	Pekin	3 cc. extract, 1:20, vein	22	Heart	1.0 × 0.6	Soft, viscid	Myxosarcoma	..	..	..	..	Grafts used for transmission
14	E	Rous	Pekin	3 cc. extract, 1:20, vein (filtered)	26	Ovary	6.5 × 6.0	Soft, not viscid	Spindle cell sarcoma	..	..	+	+	Smaller tumors in lung, intestine
15	A	Rous	Pekin	3 cc. extract, 1:20, vein	120*	Wing	13 × 4.5	Soft, not viscid	Spindle cell sarcoma	+	+	−	−	Source of strain A
16	S1	Rous	Pekin	3 cc. extract, 1:20, vein	217*	Spleen	5.0 × 4.0	Soft, not viscid	Lymphoblastoma	−	−	−	−	Animals still alive
17	E	Rous	Pekin	3 cc. extract, 1:20, breast (filtered)	113*	Liver (No breast tumor)	0.6 × 0.4	Soft, not viscid	Sarcoma-lymphoblastoma	+(filtered)	+	−	−	Source of strain E

* Duck killed.
† Also hemorrhagic disease in chicks after intravenous injection of 1 cc.

venously at the age of 1 day with 4 cc. of filtered extract of Rous sarcoma. The growth was a small tumor, histologically a giant cell sarcoma (Fig. 2), apparently arising from the bone of the face. It was found when the animal was killed 40 days after the injection and was in all probability a late lesion.

The results concerning the transmissibility of the duck tumors to adult chickens, to chicks and ducklings are also summarized in Table III. The animals injected in the different groups have been listed by their age which, excepting duck No. 6 of experiment H, is the same as the age of their tumors. The material for each injection was tested by way of the vein of the breast on a minimum of 3 ducklings, 3 to 5 days old, and 2 chickens. Chicks were occasionally injected.

It is clear from Table III that extracts from immediate tumors (virus or cell-induced tumors which grew in ducks for 30 days or less) did not induce tumors in ducks whereas they effectively induced tumors in chickens or hemorrhagic disease in chicks. It will also be noticed that most of the immediate duck tumors had the gross and microscopic features of the original Rous sarcoma. An exception to this rule was duck tumor H, which differed from the others in that the animal was 4 days old when injected and showed metastases at death. It was because of this deviation that transmission was attempted.

On the other hand, extracts from the late sarcomas from ducks Nos. 15 and 17 of experiments A and E (which grew in their hosts for 121 and 113 days, respectively) were active for ducks but not for chickens. Lymphoblastoma of duck No. 16 of experiment S1 has not been successfully transmitted to either. These 3 tumors and the giant cell sarcoma of experiment E had features which distinguished them completely from the original Rous tumors. Extreme youth of the host and large virus dosage, factors indispensable for the infection of the first generation of ducks, were no longer required for the infection of the second generation. All of the 14 ducks injected with filtrates of tumors A and E (from ducks 15 and 17) were from 3 to 5 days of age, and the amount of extract injected was only 1 cc. Yet all developed rapidly growing generalized tumors. Furthermore, the route of infection was no longer an important factor, since the extracts were equally effective when injected in the vein or in the skin.

Table III also shows that transmission by cell suspension was easy for all tumors regardless of their age. Following the inoculation of 9 out of 11 immediate and late tumors, 20 out of 30 ducks injected developed tumors. Transmission failed with 3 immediate tumors when grafting was resorted to. The special conditions of transmission of tumor E of duck No. 17 will be taken up later.

The results of experiment E warrant special attention. Here ducks of the same batch, infected with the same filtrate of Rous virus, developed the following varieties of tumors: spindle cell sarcoma of the ovary; giant cell sarcoma of the bone; and a sarcoma-lymphoblastoma of the liver. Moreover, other ducks of the same series developed a typical hemorrhagic disease, and the few tumors accompanying the hemorrhagic lesions were myxosarcomas. Therefore, 5 varieties of lesions were induced by the same virus preparation.

Study of the Duck Strains of the Rous Tumor

As indicated in Table III, tumors from 5 ducks gave origin to 5 strains of duck tumors after serial passage through ducks of the same or other breeds. Strain H was split into strains HC and HV. The latter strains were the most thoroughly studied.

In Fig. 3 are expressed the conventional signs which will be used in the graphic representation of the passages of the strains

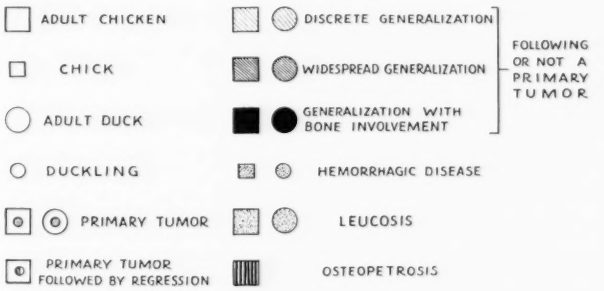


Fig. 3.—Conventional signs used in transmission diagrams and descriptive charts. See text.

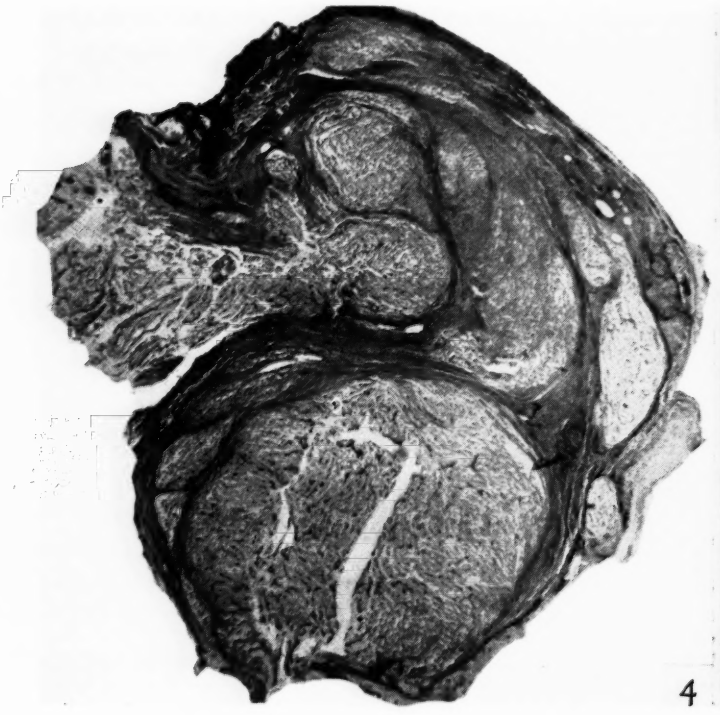
either in ordinary or in three dimensional charts. In some cases signs representing two different sorts of lesions will be combined in the same individual. The small figure underneath the signs indicates the number of days subsequent to injection at which the animal died or was killed. Some charts contain additional pertinent indications. With a few exceptions tumor extracts at 1:20—filtered or not—and cell suspensions at 1:5 were employed for transmission, the former being injected in the vein and the latter in the breast. Both materials were prepared in saline solution and 1 cc. was injected routinely.

Strain A.—The source tumor (No. 15 of Table III) developed in the wing of a Pekin duck injected intravenously within the first 24 hours after hatching with 3 cc. of unfiltered extract of Rous tumor diluted 1:20 with saline. The animal was killed 120 days after injection, and a large, rapidly growing spindle cell sarcoma composed of nodules of cells arranged in whorls and separated from each other by large bands of collagenous tissue (Figs. 4 and 5) was found.

The tumor was carried through two more generations of ducklings, all of which developed a generalized disease. Following this, passages were purposely discontinued. A graphic summary of the tests carried out on ducks and chickens is given in Fig. 6. The gross and microscopic lesions induced

in ducklings of the 2nd and 3rd passages were identical with those induced by strains HV and HC to be studied later, and will therefore be described with them. It will suffice to say here that filtrates injected in the vein induced widespread tumors, together with hemorrhagic lesions, mostly in the skin and digestive tract, but also in the skull. Cell suspensions injected in the breast induced large collagenous tumors followed by the same generalized lesions. Death occurred from 10 to 27 days after injection.

Of the 17 adult chickens injected in the breast with extracts or cells from the 2nd and 3rd passages, 10 were found resistant, 4 developed slowly growing sarcomas which later regressed, and 1 developed a progressively growing sarcoma which was followed by generalization. The two other chickens died 17 and 100 days after injection. The former showed signs of an acute infection and the latter presented a large liver in which microscopic examination disclosed a very heavy periportal infiltration by lymphoblast-like cells. Chicks injected in the breast with cells developed rapidly growing but localized and well encapsulated tumors, which killed their hosts in 2 to 3 weeks. Those injected with filtrates in the vein showed no lesions when killed 22 days later.



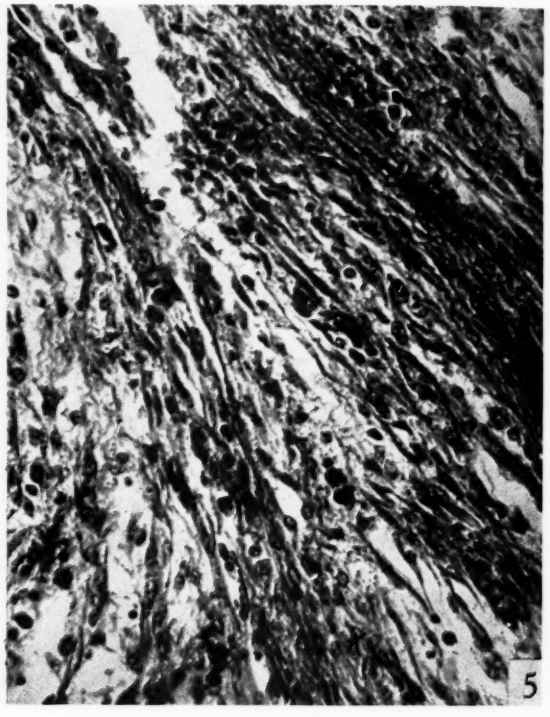


FIG. 4.—Collagenous tumor from the wing of a duck injected intravenously 1 day after hatching with extract of Rous tumor and killed 4 months later. Source of strain A. Masson stain. Mag. × 20.

FIG. 5.—Section of the same tumor as in Fig. 4. Mag. × 300.

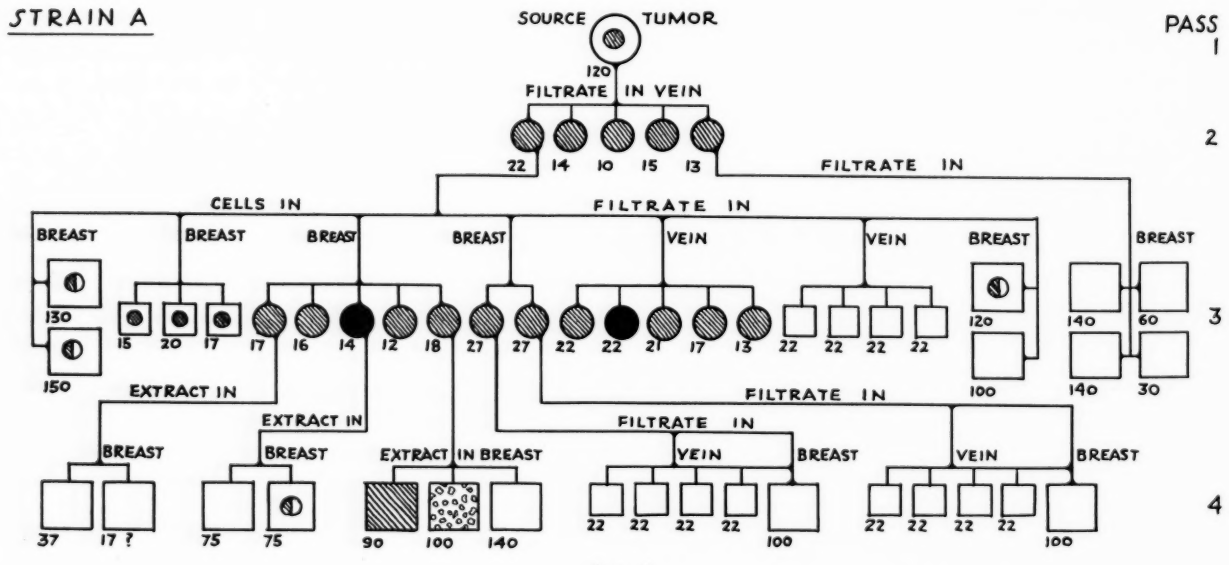


FIG. 6

Comment.—Despite the incompleteness of the study it is clear that the source tumor bred true histologically or in rate of growth. It is also clear that the ability of the virus to induce immediate sarcomas in chickens and chicks was to a large extent lost. Analysis of the data indicates that the possibility exists that the virus induced lymphomatosis in one chicken.

Strain E.—The source tumor (No. 17 of Table III) was a small growth that was found buried in the liver of a Pekin duck killed 113 days after injection with 3 cc. of filtrate of Rous tumor 1:20 in the breast 24 hours after hatching. There was no breast tumor. The liver growth was soft, not viscid, and well encapsulated, and the pancreas showed several small, flat, neoplastic nodules.

and that others developed an acute disease of doubtful etiology (6th passage B) may have had some influence on the results. But it may well be that, as is often observed in tumor transplantation, something inherent in the tumor or virus itself was the cause of the failure.

No histological study was made of the primary tumor because it was used in its entirety for transmission. However, the nodules from the pancreas consisted of zones of fibroblastic cells surrounded by a ring of lymphoid and lymphoblastic cells. The same mixed lesions were observed in the passages either in the viscera (Figs. 8 to 12) or in the skin in the only case where a tumor developed in this organ. Moreover, nodules of lymphoid cells such as those occurring in lymphomatosis, without any fibroblasts, were present in viscera (Fig. 12) while in one duck of the 6th passage a tumor consisting of sarcoma cells was observed in one adrenal (Fig. 11). Polymorphonuclears and myelocytes either scattered or in a small cluster were also present in the mixed lesions.

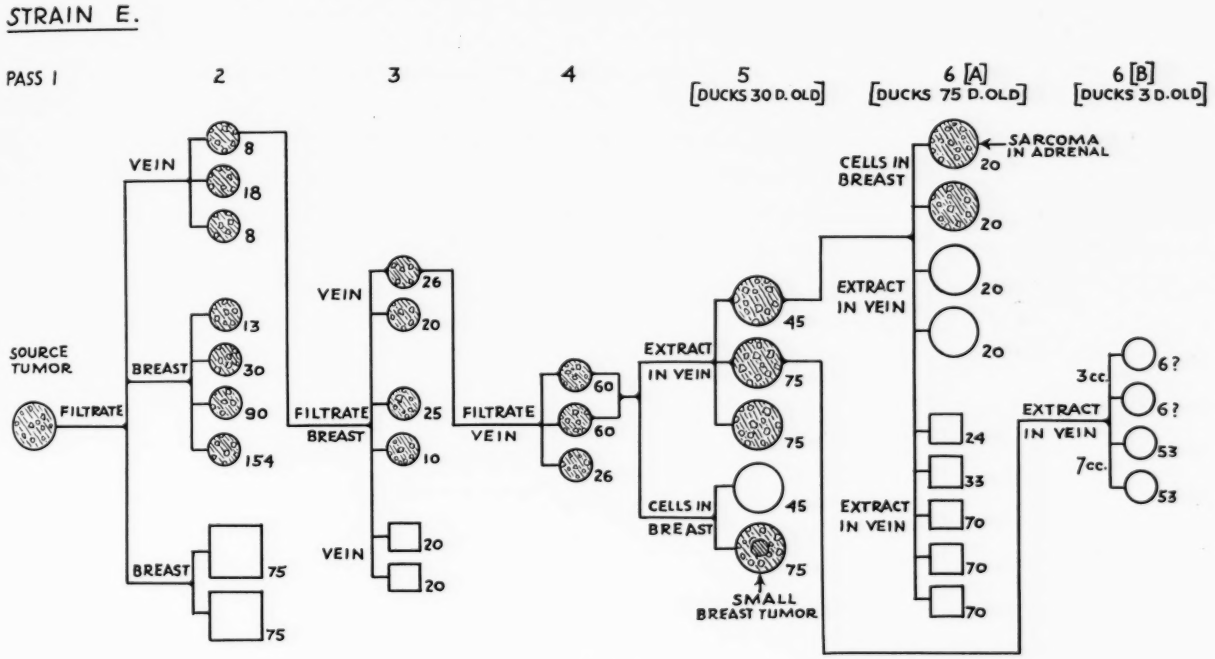


FIG. 7

A summary of the passages is given in Fig. 7. Throughout the passages intravenous injections of tumor filtrates induced a disease affecting the spleen in every case. The liver was frequently affected while the lung, intestine, and pancreas showed only occasional lesions. An adrenal tumor was found in one instance. The organs heavily involved were studded with whitish, soft, nonviscid nodules from less than 1 mm. to 7 mm. in diameter and had rather diffuse boundaries. Throughout the passages, filtrates or cell suspensions injected into the breast never induced a local disease except in one duck. This bird was one of 11 ducks used in the 5th passage. Eight ducks in other passages injected in the breast developed visceral lesions. With few exceptions the tumors grew very slowly. Passage ducks rarely died of the tumor growths, but were sacrificed from time to time for passage material. However, in a few ducks killed as soon as 8 days after intravenous injection, small growths had already appeared in the spleen. Filtrates were ineffective in inducing lesions in chickens or chicks. The strain was lost at the 6th passage due to obscure reasons. The fact that some of the ducks were killed too soon (6th passage A)

Comment.—The tumor bred true (a) histologically, (b) in its rate of growth, and (c) in its inability to induce local growths, the subcutaneous route being as effective as the intravenous one in producing typical visceral lesions. The tumor, without any doubt, contained both sarcomatous and lymphoblastomatous elements, one of them occasionally taking the upper hand with complete exclusion of the other. Whether these cells were independent or derived from each other cannot be decided. The point of interest, however, is that the Rous virus has given rise to both types of neoplastic growth in the same animal. The poor transplantability of the tumor would be in line with what is known about the difficulty in transmitting lymphoblastomatous tumors. It is pertinent to recall in this connection that the lymphoblastoma from the

spleen of duck No. 16 of Table III has not so far (6 months after injection) proved to be transmissible.

Strain Z.—The source tumor (No. 5 of Table III) was a myxosarcoma induced in a Khaki Campbell duck by the injection of 4 cc. of cell suspension of Rous tumor at 1:20 within the first 24 hours after hatching.

A summary of the passage carried out is given in Fig. 13. There were indications of generalization at the 4th passage when a metastasis in the spleen was found in a duck injected with cells in the breast without developing a local tumor. At the following passage another spleen tumor was observed in a duck bearing a cell-induced primary tumor in the breast. However, 4 more ducks injected intravenously with extract of the source tumor developed a very pronounced generalized hemorrhagic and neoplastic disease affecting primarily the digestive tract, lungs, liver, spleen, and yolk sac. Microscopically the source tumor proved to be a very loose myxosarcoma, whereas the tumors of the passages were ordinary spindle cell sarcomas. The hemorrhagic lesions were identical to those in-

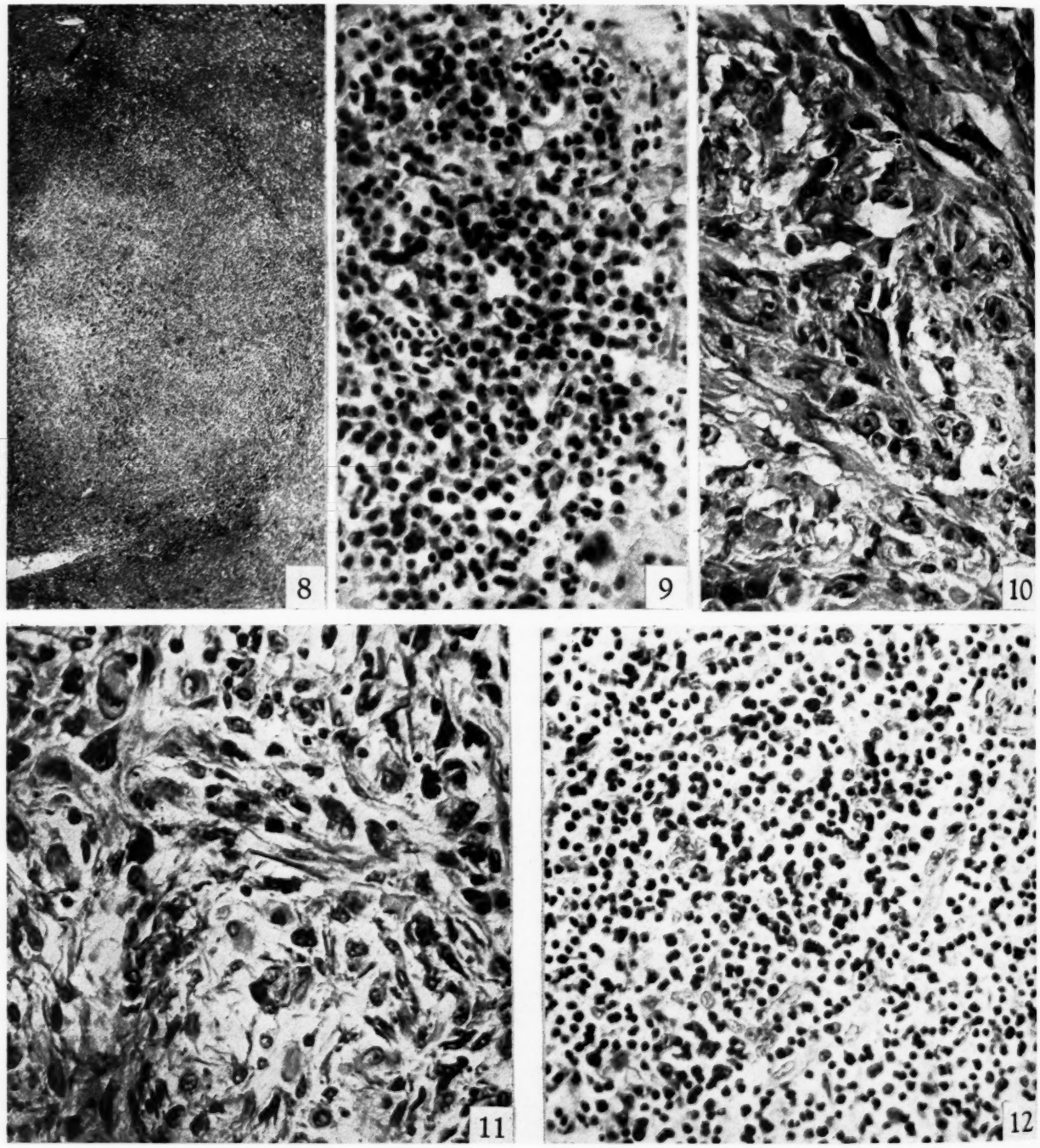


FIG. 8.—Lesion in the liver of one of the passages of strain E. Mag. × 40.
FIGS. 9 AND 10.—Peripheral and central parts respectively of the lesion shown in Fig. 10. Mag. × 450.
FIG. 11.—Sarcomatous lesion in the adrenal of a duckling from one of the passages of strain E. Mag. × 450.
FIG. 12.—Lymphoblastic lesions in the liver of a duckling from one of the passages of strain E. Mag. × 400.

duced by the Rous virus in ducklings. The series was purposely stopped at the 5th passage.

Comment.—Since experience gained with the other strains obtained by serial cell passages has shown that the occurrence of generalization is a clear indication of the transformation of the chicken strain into a duck strain, we believe that such transformation has taken place in the present case. In view of the failure of Purdy (26) to infect Khaki Campbell ducklings with extracts of Rous tumor it is pertinent to point out that generalization in our strain was obtained in Pekin ducks since Khaki Campbells were not available at the time.

Strain V.—The source tumor (No. 7 of Table III) was a spindle cell sarcoma induced in a Puddle duck by the injection in the breast of 4 cc. of cell suspensions of Rous tumor at 1:20 within the first 24 hours after hatching.

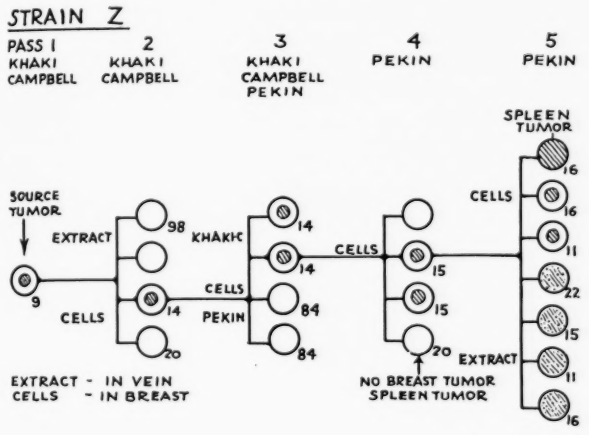


FIG. 13

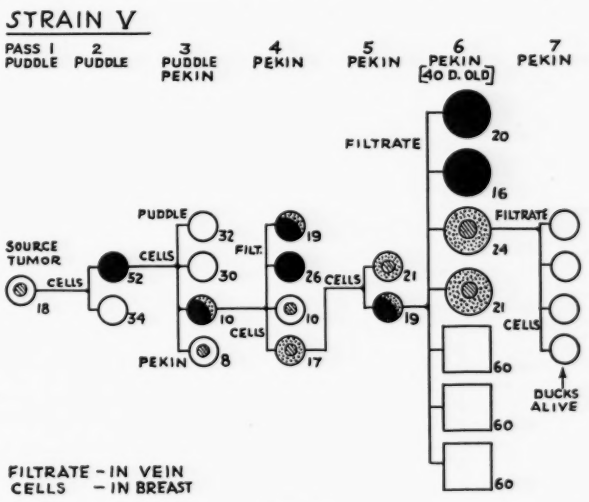


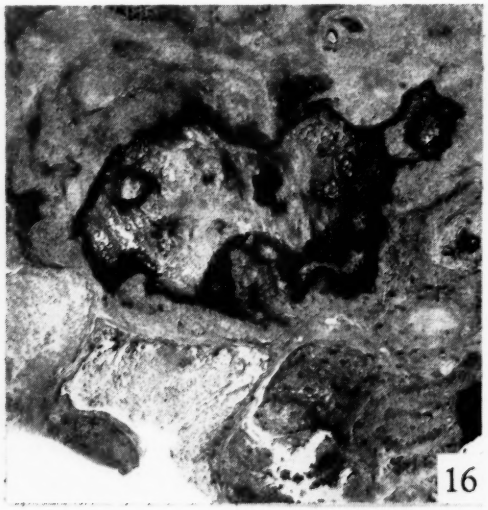
FIG. 14

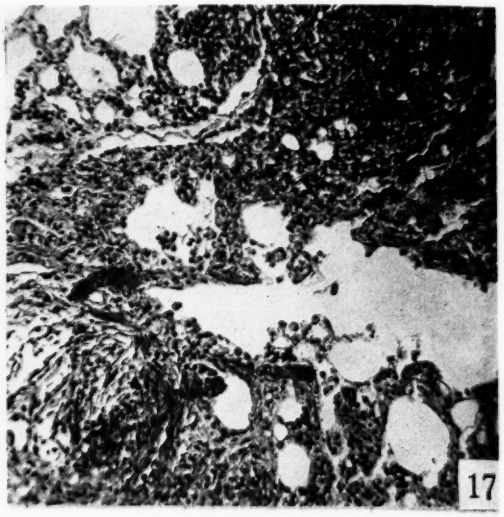
The results of the passages carried out are given in Fig. 14. The primary growths were very characteristic. They consisted of a tumor flatly spread over the breast integrated by small round nodules of different sizes separated by a stroma. Some areas of the tumor were viscid. The generalized lesions produced either by metastasis of the primary growth or by intravenous injections of filtrates were of two main types: 1. hemorrhagic lesions in viscera and bone marrow, and 2. periosteal and endosteal tumors. These lesions appeared separately or combined. The hemorrhagic lesions, most numerous in the liver and spleen, consisted of blebs from a few millimeters to several centimeters in diameter. These sometimes dissected the whole organ because of their large size. No association of these lesions with tumor tissue was grossly found in any case. Periosteal tumors were sometimes solitary in the affected bone, were thinly spread over a good length of it, or were multiple. In the ducks of the 6th passage practically every bone showed multiple tumors which gave a typical radiological image (Fig. 15).

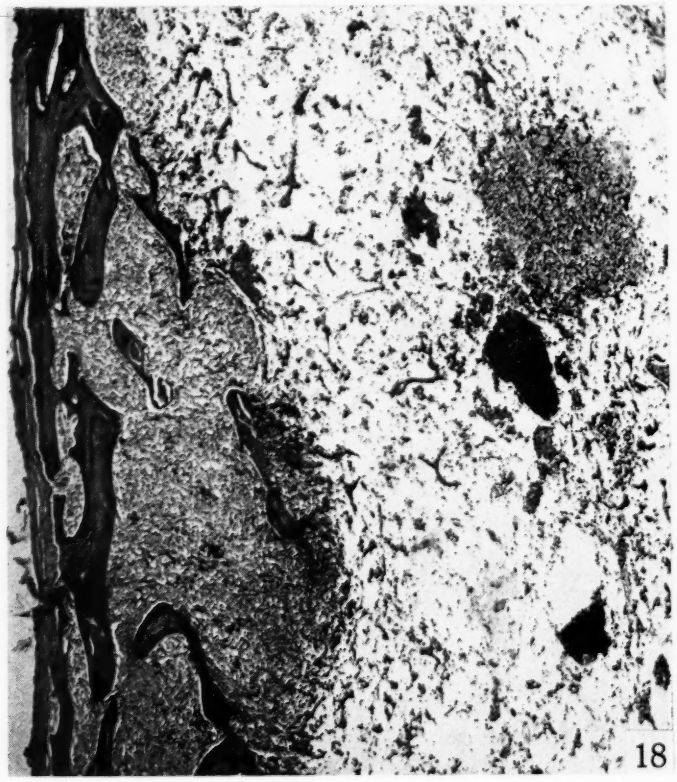
Microscopically the nodules composing the primary tumors consisted of whorls of fibroblastic cells surrounded by delicate strands of collagen. The presence of these collagenous bands together with the necrotic areas gave the tumor a very typical appearance (Fig. 16). In some cases there was a conspicuous proliferation of new vessels. Microscopically the visceral blebs again showed their independence from neoplasia, and a more complete study of them will be reported elsewhere. The tumors of the soft tissues were composed of either typical spindle-shaped fibroblasts or of sheets of large round cells. In the lung both types of tumors were observed side by side (Fig. 17). Within the marrow cavity were collections of tumor cells in sheet-like arrangement. These cells possessed a large amount of pale cytoplasm which at times appeared to be vacuolated. The periosteal and endosteal tumors were not accompanied by significant new bone formation (Figs. 18 and 19); on the contrary, osteoclastic activity as well as necrosis of trabeculae gave evidence of bone destruction.

Comment.—Changes in the disease suggesting transformation of the chicken strain into a duck strain; *e. g.*, development of a bone tumor and special pattern of the primary tumors, occurred at the 2nd passage. Although it is quite possible that the variety of ducks used may have enhanced this transformation, it should be pointed out that the tumors grew in the ducks of the 2 first generations for a total of 70 days. The ability to induce both peri- and endosteal tumors and pure hemorrhagic lesions, even in ducks 40 days old when injected (6th passage), were distinctive traits of the strain. Passages are still being continued, and a complete description of the results will be given in another publication.

In summary, despite the fact that only an incomplete investigation was made of the strains of duck tumors obtained, one can state that each of them showed typical and constant characteristics whereby they could easily be differentiated from the others. In the 3 strains where the point was studied, a common negative characteristic was found; namely, the inability shown by extracts of the tumors to induce, in most instances, sarcomas in adult chickens. In the 2 other strains which will be described, a thorough study was made of the disease in ducks as well as in chicks. The study







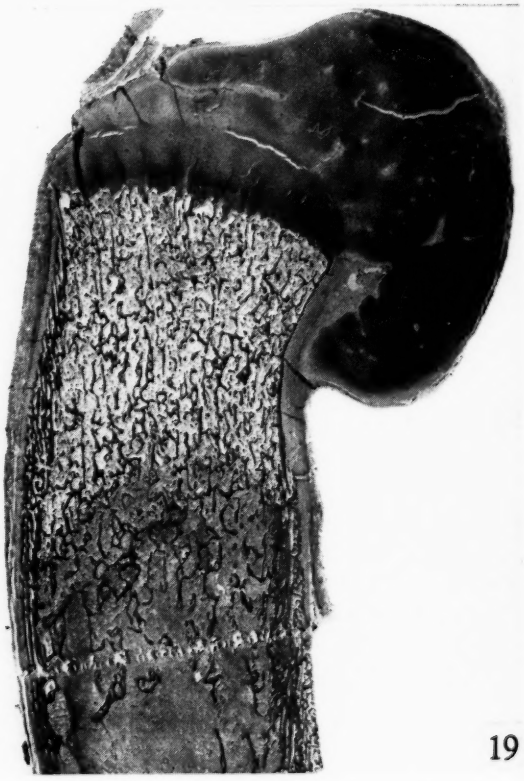


FIG. 15.—Radiograph of the radius and ulna of a duck of the 6th passage of strain V, showing multiple tumors in the bones.

FIG. 16.—A primary tumor in a duckling of the 3rd passage of strain V, showing pattern produced by zones of necrosis within the tumor. Masson stain. Mag. × 13.

FIG. 17.—Tumor nodules in the lung of a duckling from one of the passages of strain V. Note the different histological appearance of the nodules. Mag. × 150.

FIG. 18.—Endosteal tumor and tumor nodule in the marrow of a duck from the 4th passage of strain V. New bone production is slight. Mag. × 50.

FIG. 19.—Sarcomatous invasion of the bone marrow in a duck of the 6th passage of strain V, same as Fig. 15, showing bone destruction. Mag. × 8.

of the disease in chicks is the basis of the second part of this report.

Strains HC and HV.—The source tumor (No. 6 of Table III) was originated by the injection of 4 cc. of cell suspension at 1:20 in the breast of a 4-day-old Pekin duck. The animal died 16 days later with a large viscid tumor in the breast and a few small nodules in the lung. Cell suspensions and extracts at 1:20 dilution of the breast tumor were obtained. The cells were passed to a second generation of ducks, and the passages were continued by this method. This is strain HC, the last letter indicating that cells only were used throughout the transplants. Two cc. of filtrate of this tumor was injected intravenously into each of 3 ducks also 4 days old. One of them developed a tumor 3.5 × 3.5 cm., plus some others of a smaller size, in the skin of the neck.[2] The duck was killed 17 days later and cell suspension of its tumors injected into the breast of other ducks induced rapidly growing tumors followed by generalization. The passages were continued by inoculation of cell suspensions. This is strain HV, the last letter indicating that virus was the cause of the first tumor of the series.

A three dimensional representation of the passages and experiments carried out with the two strains is given in Figs. 20 and 21. The description that follows refers to the disease observed in strain HV since the 2nd passage, in strain HC since the 9th passage, and also in strain A. The lesions were identical in all the strains although their incidence varied.

The primary tumors were firm, resilient, translucent, showed bands of necrosis, and had a very characteristic structure (Fig. 22). Necrosis increased in the later passages and this resulted in the formation of cavities and less cohesion of the growth. Viscidity, so characteristic of the original Rous tumor, was absent or very scant in these tumors, and this indicates a profound functional difference between the malignant fibroblasts of chicken and duck tumors.[3] The distinctive features of the generalized lesions were the involvement of the skin, whole digestive tract, and bones (Fig. 23). The involvement of the skin and that of the preventriculus and small intestine was frequently massive. That of the skull and ribs was moderate. Tumors in the long bones were in most cases symmetrical, and in a few cases generalized to all the extremities (such tumors were not observed in strain A). Metastases in the spleen, liver, and lung were frequently found while metastases in the muscles, heart, kidney, pancreas, trachea, peritoneum, etc. occurred less frequently. The tumors in viscera differed widely in size and if they were large enough one could recognize in them the typical gross pattern of the primary tumors. Viscidity was observed in some of them, whereas bone tumors never showed it.

A curious occurrence only occasionally met with was the development of solitary tumors in different parts of the body in the absence of primary tumors. One of these, a fungating sarcoma in the auditory canal, is reproduced in Fig. 24. It developed in a duck injected at the age of 17 days with cells of the 3rd passage of series V. The bird was killed 7 weeks after injection. The way these tumors were induced can be compared to those induced by the original Rous virus injected into newborn ducks.

Hemorrhagic lesions similar to those shown in Fig. 23, developed in the same locations where tumors were found. In many cases the blebs were seen grossly free of neoplastic tissue, an observation confirmed by microscopic study. The findings of large blood clots surrounding the liver, of free blood in the peritoneal cavity, and the replacement of the spleen by a large clot were a common occurrence. Hemorrhagic lesions were most frequently observed in strain HC, and, in some ducklings injected with this strain, they developed with complete exclusion of secondary tumors. Another important finding was the great hypertrophy of the liver and spleen in the absence of neoplasia or hemorrhage within these organs, and occasionally, in older ducks, in the absence of generalized lesions.

Microscopically the source tumor lacked any special pattern, and consisted of large fibroblasts loosely arranged much as in the same tumor growing in chickens. The lung metastases had the same structure as the primary tumor but some of the growths were surrounded by a ring of actively dividing lymphoblastic cells very much like the lesions of strain E.

After the tumor had become adapted to the ducks, the primary growths showed a very typical pattern, much the same as in Fig. 16: whorls and nodules of fibroblasts separated by bands of collagenous tissue alternating with zones of necrosis. The generalized lesions were also spindle cell sarcomas with a varying degree of cellularity; in the intestine they were mostly subserosal. In the bones they were periosteal and endosteal with little or no proliferation of new bone (Fig. 25). Giant cell sarcomas were sometimes observed. Some of the tumors were largely collagenous and one could detect in them the same pattern as in the primary tumors, while in other cases they contained a large number of hemorrhagic foci (Fig. 26). In the liver, in those cases showing hypertrophy there was a pronounced vacuolization of the cord cells, and a discrete periportal infiltration of lymphoblastic cells actively multiplying.

Separate tests showed that filtrates in the breast induced the same local and generalized disease as when cells were injected, and, if introduced into the vein, the same generalized lesions as those secondary to breast injections followed. This suggests that in young ducks, as in young chickens injected with the original Rous tumors, metastases may be induced by virus as well as by cells.

In the course of the passages it was observed that the age of the duck was in inverse relation with the intensity of the disease, coinciding with an analogous finding with chickens injected with the Rous virus (4). In 8 experiments in which virus from both strains was injected in proportionate amounts into ducks from

[2] The frequency with which tumors developed in the anterior part of the neck skin in ducklings injected with tumor viruses which may well be called dermotropic is due to the localization of blood-carried virus in an area which is constantly subjected to friction while the animal feeds. This was very frequently observed in strains A, HC, and HV.

[3] The viscidity of the Rous sarcoma, and other tumors as well, is due to the secretion by the fibroblasts of a mucin largely composed of hyaluronic acid which is the substrate acted upon by the spreading factors from tissues and other sources. This mucin is analogous to that existing between normal fibroblasts, in synovial fluid, etc. The findings concerning the changes from mucin to collagen may be of interest from the point of view of the histogenesis of the latter.

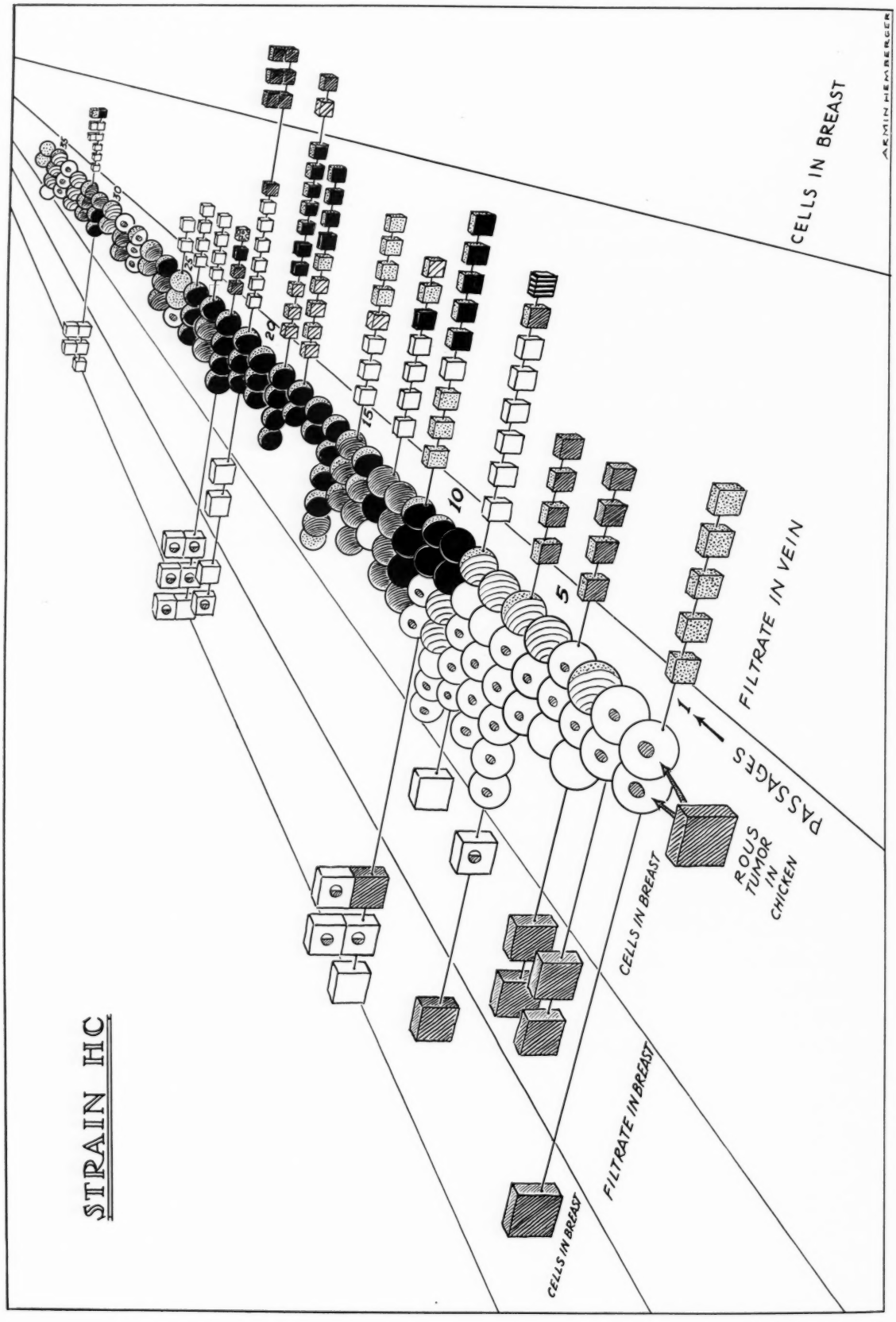
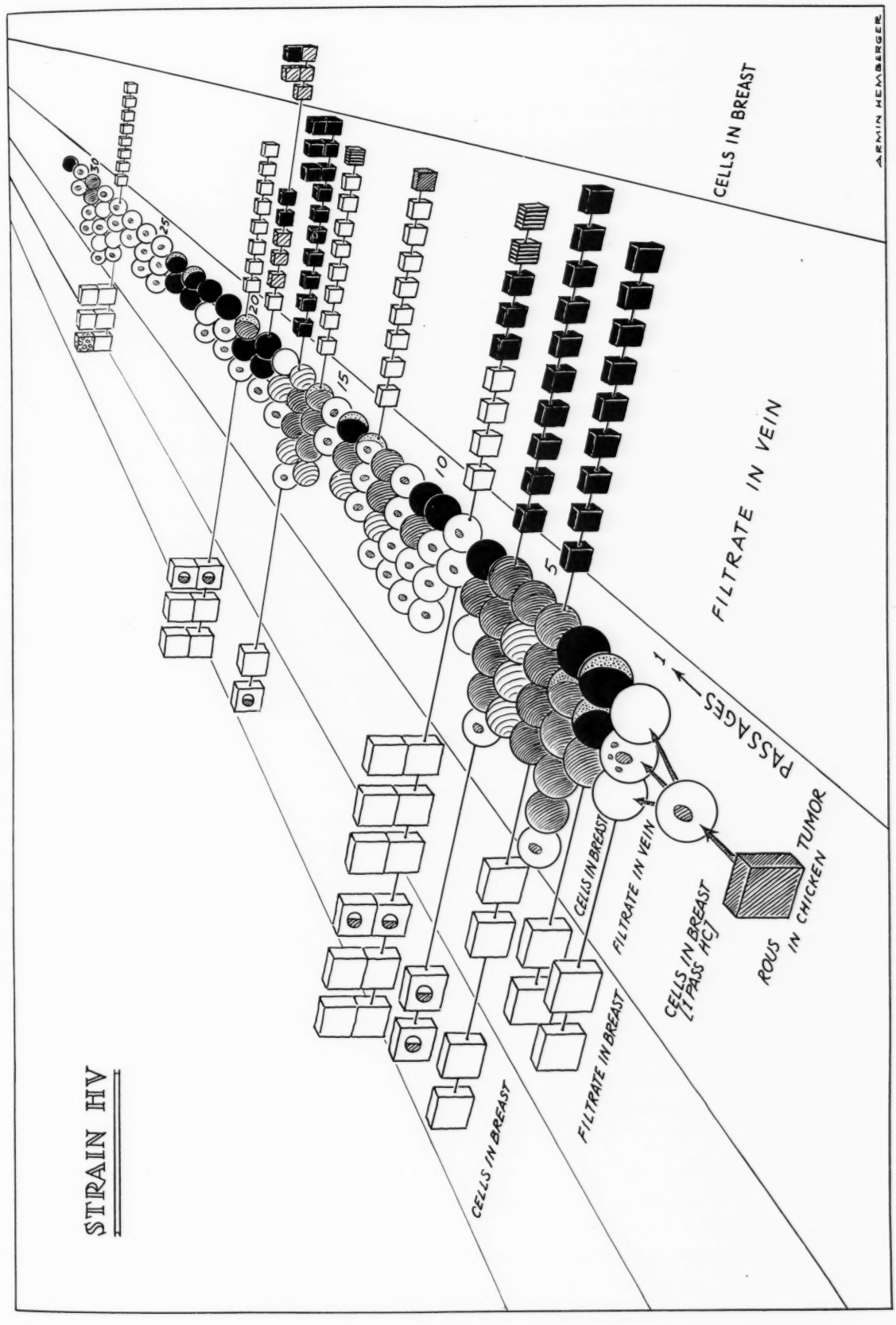

STRAIN HC
CELLS IN BREAST
FILTRATE IN VEIN
PASSAGES
1
5
10
15
20
25
ROUS TUMOR IN CHICKEN
CELLS IN BREAST
FILTRATE IN BREAST
CELLS IN BREAST
ARMIN HEMBERGER

FIG. 20


ARMIN HEMBERGER
CELLS IN BREAST
FILTRATE IN VEIN
PASSAGES
1
5
10
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20
25
30
TUMOR
IN CHICKEN
ROUS
CELLS IN BREAST
[1 PASS HC]
FILTRATE IN VEIN
CELLS IN BREAST
FILTRATE IN BREAST
CELLS IN BREAST
STRAIN HV

FIG. 21

1 day to 3 months of age the point was confirmed. The rare resistant individuals found were in general older than 2 months. On the contrary, death occurred in most ducks from 10 to 20 days after injection regardless of the presence or absence of generalization, those with a single primary tumor dying just as fast as those with widespread metastases. Death was not attributable to bacterial infection as cultures showed.

ducks, and tumor cells from each passage were used to secure the next, the primary tumors underwent a progressive change from the noncollagenous, viscid, soft chicken type to the collagenous, nonviscid, firm duck type. Typical examples of such tumors were irregularly observed in the 9th and 10th passage but constantly observed from the 11th passage on. Generalization was also progressively accomplished; one metastasis in the spleen appeared in one duck at the 5th passage; a few skin blebs in another duck of the 6th passage; discrete intestinal tumors in another of the 7th; and widespread lesions with involvement of the ribs at the 9th. However, invasion of the liver, probably by direct extension of the breast growth, was frequently observed from the 2nd passage on.

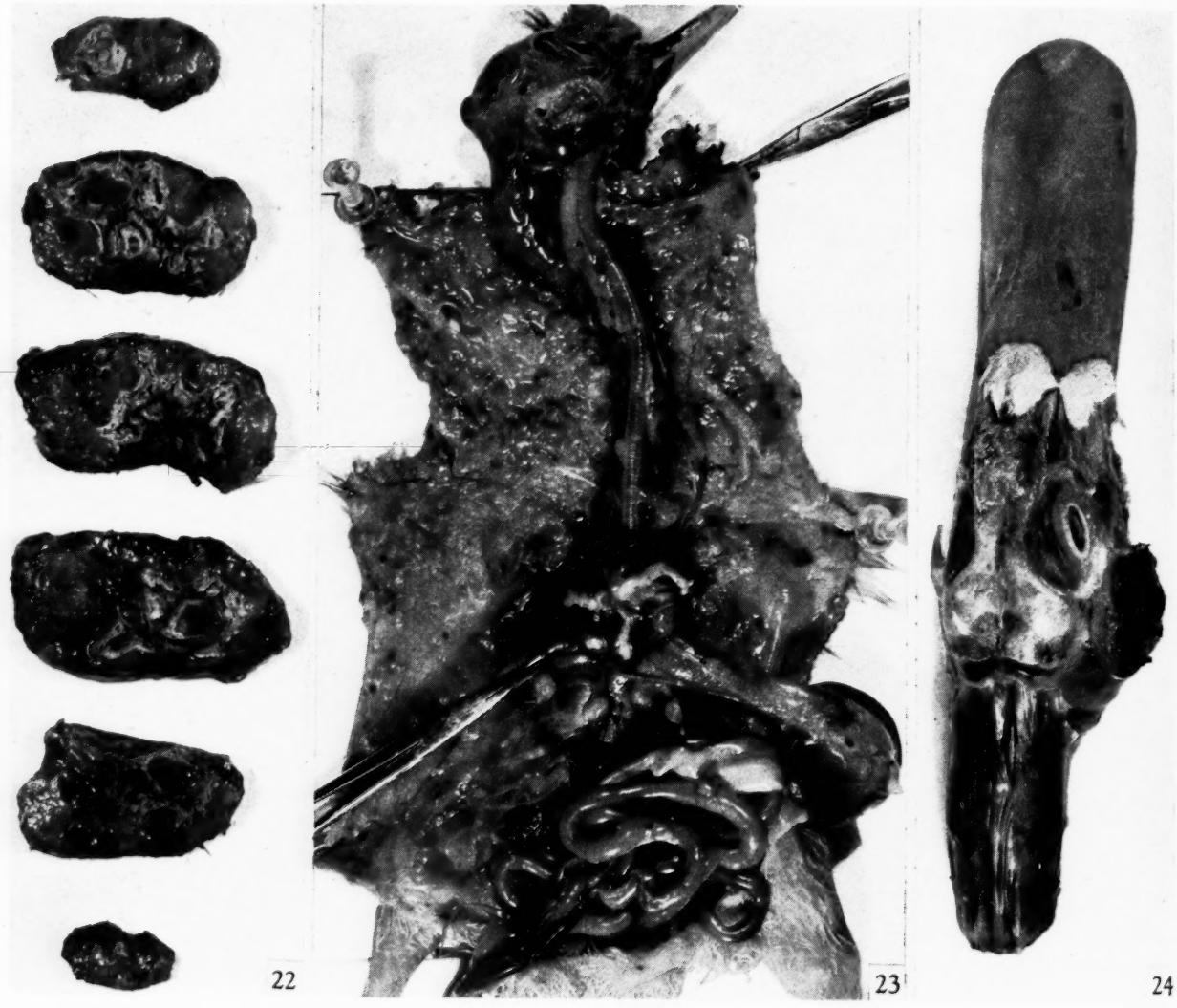


FIG. 22.—Series of sections through a tumor of a duck from the 6th passage of strain HV. Note area of ulceration and underlying necrotic tissue.

FIG. 23.—Generalized disease in a duckling injected intravenously with virus from the 2nd passage of strain HV.

FIG. 24.—Sarcoma in the auditory canal in a duck injected in the breast with a cell suspension from a tumor of the 3rd passage of strain HV. No local tumor developed.

Comment and additional experiments.—On comparing strains HV and HC in Figs. 20 and 21, one notices first the differences in the occurrence of primary collagenous tumors and of generalized lesions according to whether filtrates in vein or cell suspension in breast was used for transmission. In strain HC where cells from a chicken tumor were injected into the breast of

On the contrary, in strain HV, a strain derived from a tumor induced by a cell-free extract, generalization took place at once at the 2nd passage in the 3 ducks injected intravenously, but typical collagenous tumors were not clearly present until the 8th passage.

Lesions in strain HC from the 9th passage on were identical to those of strain HV but, as partly shown in Figs. 20 and 21 their incidence was different. The most important differential trait between the two strains was the pronounced hemorrhage-inducing such as tumors arising from bones, enlarged livers, intestinal tumors, etc., appeared suddenly in one duck; on further passage the same tumor would develop in each of several successive generations, disappear, and sometimes reappear in subsequent passages. Figs. 20 and 21 present data illustrating the occurrence of bone tumors and hemorrhagic lesions. An additional experiment with the virus of strain HC supplied the best example of the phenomenon. Until the 25th passage no hemorrhagic lesions in the bone marrow were

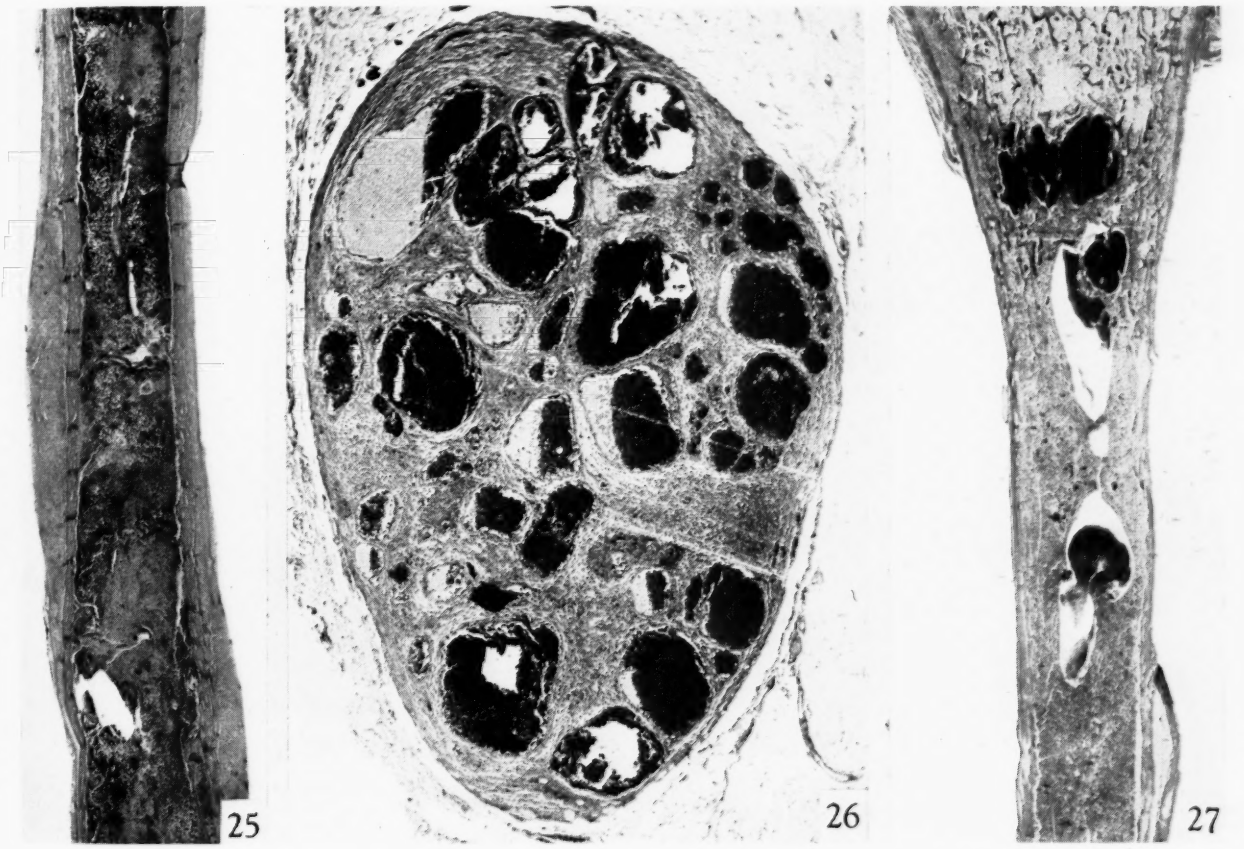


FIG. 25.—Periosteal sarcomas in a duck of the 16th passage of strain HV. Note the absence of bone proliferation. Mag. × 9.

FIG. 26.—Hemorrhagic skin tumor of the 2nd passage of strain A. Mag. × 40.

FIG. 27.—Hemorrhagic foci in the absence of tumor in the bone marrow of a duckling from the 25th passage of strain HC. Mag. × 11.

property of strain HC as compared to that of strain HV.[4] Proper study can thus make apparent differences in two series of tumors even when they are derived from the same source.

That changes occurred in the virus during transplantations is suggested by the fact that a given lesion observed in ducks of any age injected with virus of this strain in the vein or cells in the breast. Then, at this passage, the tumor was implanted in the breast of Puddle ducks 24 hours old. The animals died 14 days later with primary tumors, pronounced neoplastic and hemorrhagic lesions in viscera, but only hemorrhagic foci in the marrow of the bones of the extremities (Fig. 27). Filtrates of the Puddle duck tumors were injected in the vein of Pekin ducks 8 days old, and exactly the same blebs developed in the marrow while the viscera showed a very pronounced hemorrhagic disease accompanied by some tumors. The tumors implanted into a second generation of

[4] Other differences concerned the incidence of lesions in the skin and digestive tract (19 per cent and 14 per cent in strain HC against 39 per cent and 29 per cent in strain HV, respectively), and in the lungs (13 per cent in strain HV against 25 per cent in strain HC). The liver was more affected in strain HC but the impossibility of knowing whether the lesions were real metastases or due to direct propagation of the growth precludes final conclusions.

Pekin ducks induced large primary growths followed again by blebs in the marrow. After this, no more blebs in marrow were observed.

On several occasions injection in other ducks of extracts from the hemorrhagic lesions, in the absence of tumors, and of the enlarged livers was carried out. No lesions of any kind were ever induced. The reasons for the failure in transmitting the hemorrhagic lesions will be considered in another paper. The failure of the liver extracts to induce any other lesions would fit with the lymphomatous character suspected in the lesions present in the organ.

Results obtained in the infection of adult chickens reflect the adaptation, sudden or gradual, of the chicken virus to the new host. Figs. 20 and 21 show that the capacity of the virus to induce immediate tumors in chickens is only progressively lost in strain HC as the adaptation to the duck is progressively accomplished; whereas, in strain HV it is totally lost at the first passage. The same holds true to a large extent when cells are injected, although in both strains there may be left in them a residual capacity to induce tumors. In strain HV these tumors always regressed and the same happened in strain HC in 11 out of 12 cases when suspensions of tumor cells of the 10th and later passages were employed. It is noteworthy that, in this strain, whenever progressively growing tumors developed, the time of death was retarded from 17 days in the first passage to 46 in the 10th when cells were injected, and from 45 days in the 1st passage to 75 in the 3rd when extracts were injected. Also, the tumors induced by materials from the early passages resembled the original chicken tumors whereas those of later passages resembled duck tumors. Passages of these tumors were attempted in 3 instances. The procedure followed and results obtained are summarized in Table IV.

The results show the absolute lack of transplantability to other adult chickens of tumor resulting from the growth of duck tumor cells in chickens. This important point will be fully developed in the following section.

TABLE IV: TRANSPLANTATION OF TUMORS INDUCED IN CHICKENS BY CELLS FROM DUCK TUMORS

Source of tumor and disease of the donor					Disease of the recipient			
Chicken number	Strain	Passage	Severity	Time of death	Tumor material used for transmission	Chicken number	Severity	Time of death in days	Type of resultant tumors
1	HC	1st	Pronounced	17 days	2 cc. cell suspension	1	Pronounced	20	Viscid; chicken type
					1:20	2	Medium	30	Viscid; chicken type
					1 to 20 cc. filtrate	3	Pronounced	25	Viscid; chicken type
					1:20	4	No tumors		
2*	HC	10th	Medium	46 days	2 cc. cell suspension	5	Medium	60	Collagenous; duck type
					1:20	6	Medium	60	Collagenous; duck type
3	HV	6th	Scant	Survived	2 cc. cell suspension	7	No tumors		
			Tumors regressed		1:20	8	No tumors		
					1 to 20 cc. filtrate †	9	No tumors		
					1:20	10	No tumors		
4	HV	8th	Scant	Survived	2 cc. cell suspension	11	No tumors		
			Tumors regressed		1:20	12	No tumors		
					1 to 20 cc. filtrate †	13	No tumors		
					1:20	14	No tumors		

* Only chicken that developed progressively growing tumors out of 5 birds injected.
† Tumor secured by biopsies at the 12th day after injection when the growth showed no obvious necrosis.

THE INFECTION OF CHICKS BY VIRUSES AND CELLS FROM DUCK TUMORS

Analysis of the phenomena attending the infection of chicks by the viruses from the duck tumor strains HC and HV to be described now showed that despite some differences, they were essentially the same as those attending the infection of ducklings by chicken tumor viruses and, accordingly, the same order in the description of the results will be followed. To all intents and purposes the duck variants of the Rous virus behaved toward chickens as if they were genuine duck tumors, but a check on this point has so far been impossible because we could never secure the latter.

The principal characteristics of this infection are graphically represented in Figs. 20 and 21. The figures show that in 24 instances filtrates (1 cc. of an extract at 1:20) from tumors of different passages were injected intravenously into 175 chicks from 1 to 3 days of age. Although not indicated in the figures, 2 ducks were similarly injected in every instance as controls of the presence of virus in the filtrates. It is clear from the Figs. 20 and 21 that the infectivity for chicks of the virus from the different passages was extremely variable. Lesions developed in 42 per cent of the animals. Yet the virus showed its usual infectivity for ducks because all of them died with generalized lesions.

Two additional sets of experiments dealt with the importance of the age of host and the dose of virus as factors determining infection. It was found that chicks 6 days old injected in the vein with the usual amount of virus were as susceptible as younger chicks similarly injected, but only 1 out of 10 chicks 9 days old developed lesions. For reasons to be given later it is important to point out that the results hold true as far as the induction of hemorrhagic or sarcomatous lesions is concerned. That such lesions were not induced in adult chickens injected with duck viruses was already mentioned. It was also found that all of 10 chicks one day old developed sarcomas when injected intravenously with 1 cc. of tumor filtrate at 1:200 but none of 5 chicks of the same age similarly injected with the filtrate diluted at 1:2000 developed lesions.

It is seen from these results that as in the chicken virus-duck sequence the age of the host, the dose of virus, and an inherent property of the latter concerning its infectiousness for the heterologous host are factors of prime importance in inducing infection.

The importance of the route of inoculation was not directly investigated. It may be said, however, that in experiments devised for other purposes, out of 34 chicks injected in the breast (skin and muscle) with 3 times the amount of virus used in the intravenous injections, the incidence of infection was also 42 per cent. The tumors were well circumscribed and were not followed by generalization. Moreover, in 6 of them regression occurred, while no regression of the tumors following intravenous injections was ever observed.

Figs. 20 and 21 also contain data pertaining to the effect of cells from duck tumors injected into chicks. The 13 animals were 1 day old and they were injected with 1 cc. of tumor cell suspension at 1:5 in the breast. They all developed rapidly growing tumors followed by generalization and death from 15 to 25 days after injection.

From some of the tumors in chicks injected with filtrates, sublines were started employing a total of 203 chicks. These animals developed lesions identical to those of the animals injected directly with filtrates or cells from the duck tumors. The description of the lesions that follows applies to both groups of animals, the total number analyzed being 391. Here again the lesions will be classified as *immediate* and *late*. The immediate lesions were observed in 232 animals from 9 to 100 days after injection, and they were either hemorrhagic or neoplastic.

The neoplastic lesions were sarcomatous, and in one instance a myeloid tumor of the ovary was present along with the sarcoma. It will be seen later that this could be considered a late lesion.

Late lesions were observed in 14 animals. They did not appear previous to 100 days after injection. These birds were sacrificed when still in good health, from 4 to 7 months after injection, and the lesions found to be lymphoblastomas and osteopetroses.

Of the 145 animals which did not develop lesions, 103 were killed from 4 to 7 months; the remaining 42, 2 months or less after injection. It is thus possible that the incidence of late lesions would have been higher if the animals had been maintained for longer periods of time.

Hemorrhagic lesions.—These occurred only in chicks injected with filtrates from strain HC. From the 1st to the 6th passage, the disease induced in chicks by virus from this strain was generalized and identical to that induced by the original chicken virus (5). The animals died 7 to 17 days after injection, and no gross neoplastic lesions were apparent. In the later passages, after the injected virus had become completely adapted to the duck, the hemorrhagic lesions were always localized in the liver, and appeared less frequently in other organs. Large clots around the liver were typically found, and in a few cases the spleen had the appearance of a clot. Frequently, blebs were the only lesions found in the affected organs or, occasionally, in the whole chick. This observation was confirmed microscopically. When tumors in either bones or viscera were present, no association of tumors and hemorrhagic lesions was apparent at gross examination. Some new features of the hemorrhagic disease of the chick induced by duck viruses will be described elsewhere. The diseased animals characteristically presented pure hemorrhagic lesions in some viscera, and tumors arising from bone.

Neoplastic lesions.—They were all sarcomas which took origin either from the bones or the soft tissues. Bone tumors were the more frequent and were a distinctive feature of the infection of chicks by duck viruses. Most were periosteal, some were endosteal, and a few were a combination of these two types. They were firm, not viscid, and not hemorrhagic. They arose from practically every bone (Fig. 28) and the most pronounced involvement was found in chicks injected with virus of the HV strain. Here bones were often exclusively involved, while this occurred but rarely with the virus of strain HC. The older bone tumors were in general larger, firmer and less widespread. A secondary formation of new bone was often grossly noticeable in these lesions. Soft tissues were sometimes invaded by growing bone tumors but at other times growth arose directly from them. Muscle involvement was second to that of bone, but tumors were rare in viscera. Of the latter, a characteristic lesion was the replacement of part of the preventriculus by tumor tissue. Large tumors in the neck sometimes de-

veloped when the tissues of the region were soiled by virus in the process of intravenous injection. All these tumors were compact, firm, and well circumscribed. Cases of diffuse malignant invasion of organs by loose myxosarcomatous tissue such as commonly occurs in chicks injected with the Rous virus were never observed. However, when tumors reached a very large size, some viscidity was present. The large growths that developed after cell suspensions were injected in the breast, were often accompanied in the same animal by tumors in bones. The earliest death attributable to tumor growth, and not to hemorrhagic lesions, was observed 15 days after injection.

One of the chicks that died 45 days after the injection showed in addition to widespread tumors arising from bones a 2 × 2 mm. growth in the ovary which proved to be a tumor composed of immature myeloid cells. The histological nature of the tumor and facts concerning their transmission to be described later indicate that we were dealing with a late lesion at the beginning of its development; the death of the animal was attributable to the bone tumors.

Microscopically the tumors in the bones were periosteal or endosteal spindle cell sarcomas like those in ducks, except that giant cell tumors were never observed. A very important additional lesion in chicks was pronounced formation of new bone by the inner layer of the periosteum much the same as observed in the physiological intramembranous ossification from the fetal periosteum. This lesion occurred, always in association with tumor growth (Fig. 29), either when the growth had pierced the periosteum and invaded the bone (Fig. 30), or in the absence of this invasion, tumor growth and bone growth being then histologically independent (Figs. 31 and 32). However, bone proliferation without sarcoma growth was never observed. In addition to the bone proliferation there was also a pronounced fibrosis of the marrow spaces frequently present which can easily be seen in Figs. 30 and 32. Both lesions were more pronounced in older than in young tumors, and it is important to point out that these lesions are the same as those described by Jungherr and Landauer (17) in the early stages of osteopetrosis. This resemblance strongly suggests that this condition as observed by us represents a further stage of these early lesions.

The tumors from muscles and viscera were spindle cell sarcomas of the ordinary type. Intravascular growth was observed only occasionally.

Leukotic tumors and leukoses.—These lesions were observed in 4 cases. In all of them there was a tumor in the ovary as a major lesion while one of the chickens showed paralysis. Two of the animals were killed 3½ and 4 months after injection while the others died.

Microscopically 3 of the tumors were of the lymphoid type consisting of either large or small lymphoblasts. The tumor of the 4th animal was composed of myeloid cells; and it was found in an animal that died 45 days after injection with widespread tumors in bones. For reasons indicated before, although chronologically this tumor falls within the group of the immediate lesions, it is considered as a typical late lesion. In all the 3 cases there was a leukotic infiltration of the viscera of varying intensity.

Osteopetrosis.—Before describing our results it is pertinent to summarize the studies of Jungherr and Landauer (17) on the subject.

These authors described the condition in 1938 under the term *osteopetrosis gallinarum*. It was associated with an epidemic outbreak of lymphomatosis;[5] the authors thoroughly reviewed related osteopathies in other animals and man (Paget's disease, osteodystrophia fibrosa, etc.) and emphasized the frequent association between some of these conditions and a variety of hemopathies. They injected 61 newborn chicks with blood, bone marrow, and lymphomatous tissue from florid cases of osteopetrosis and obtained in the course of 4 passages 6 gross lesion cases of osteopetrosis, 6 gross lesion cases of osteopetrosis, 6 gross lesion cases of osteopetrosis associated with lymphomatosis, and 23 cases of lymphomatosis. The animals were kept for 250 days, and those that became infected did not die until from 100 to 189 days after injection. Stubbs and Furth (29) also observed bone changes resembling those of osteitis fibrosa in chickens infected with erythroleukosis virus.

In our experiments the condition was observed in 10 of the animals, 7 of which were injected a few days after hatching, 2 at the age of 4 months. The disease was the same as described by Jungherr and Landauer (17) being characterized by diaphyseal thickening and hardening mostly of the wing and leg bones (Fig. 33) with total or partial occlusion of the marrow cavity. In the florid cases the affected areas showed increased surface temperature, and the periosteum was thickened and hemorrhagic. In no case was there evidence of sarcomatosis either in the bones or elsewhere. Parathyroids were not enlarged. The gross lesions were not clearly noticeable until from 4 to 7 months had elapsed after the animals were injected.

Because of the number of experiments involved, the vagaries of the diseases, and because the chickens were not kept long enough, it is hard to estimate from our data the incidence of leukoses and osteopetrosis. If calculated on the strict results obtained, the incidence would be 3.5 per cent.

[5] The outbreak occurred in Connecticut in the years 1933 and 1934. The chickens showed big liver disease, paralysis, and still other manifestations of lymphomatosis. There were 70 recognizable cases of osteopetrosis among 4,500 chickens from 7 strains, while many other chickens showed slight bone changes. The earliest lesions were noticed at the age of 6 weeks. Only 5 sporadic cases of osteopetrosis were observed in the routine autopsies carried out during 7 years and comprising 16,949 chickens.

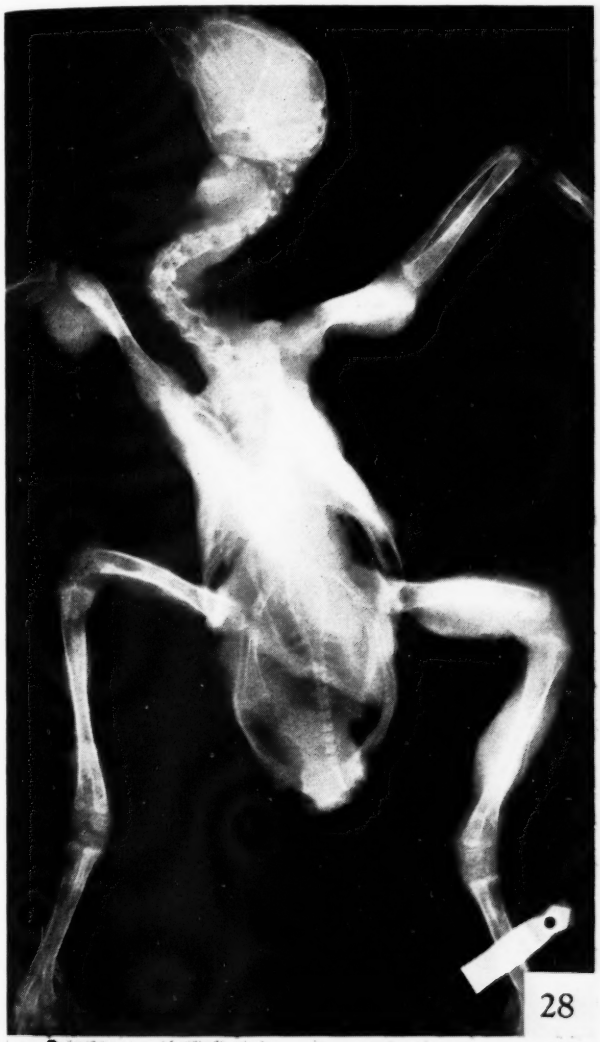
DESCRIPTION OF FIGURES 28 TO 31

FIG. 28.—X-ray picture of a chick injected intravenously with filtrate from a duck tumor of the 4th passage, strain HV. Note periosteal tumors in almost every bone. Viscera were wholly free of tumor.

FIG. 29.—Sarcoma in the thoracic wall of a chick injected intravenously with a filtrate from a tumor derived from the 4th passage of strain HV. Note subperiosteal bone proliferation in the rib surrounded but not invaded by sarcoma, and absence of bone proliferation in adjacent rib not lying within the tumor.

FIG. 30.—Invasion of bone by periosteal sarcoma, pronounced formation of new bone, and fibrosis of marrow spaces in the tibia of a chick injected intravenously with virus from a tumor derived from the 4th passage of strain HV. Note original dense cortical bone. The animal died 44 days after injection. Mag. × 17.

FIG. 31.—Humerus of the same chick. Note pronounced formation of new bone and fibrosis despite the fact that the bone was not invaded by the tumor. Mag. × 12.



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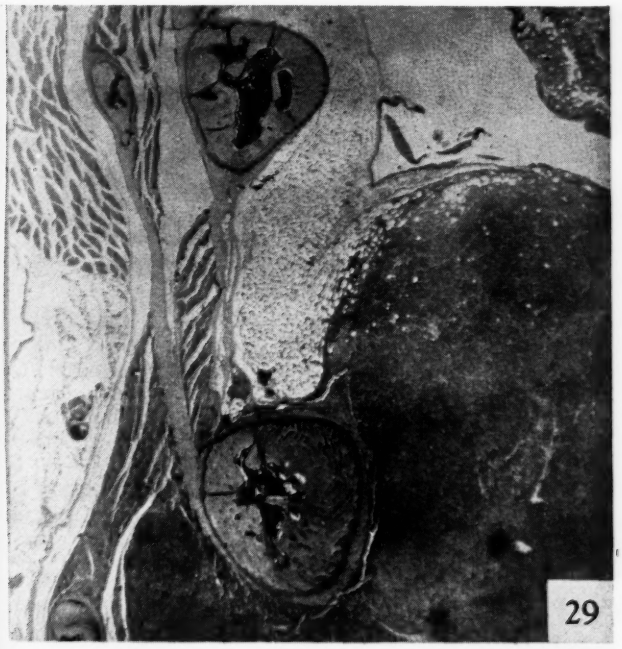

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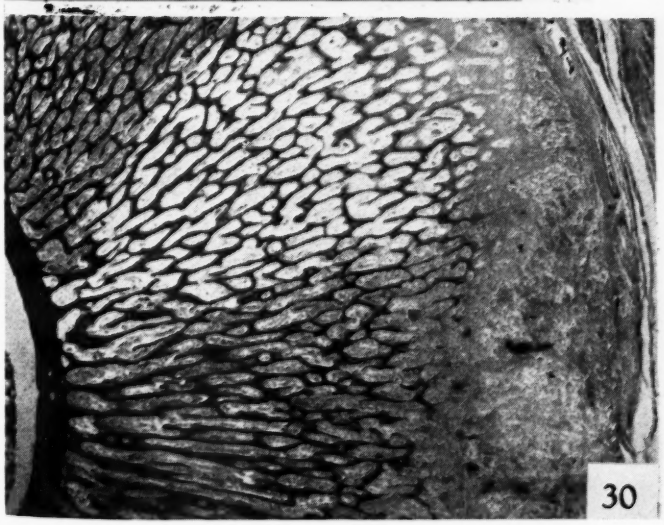

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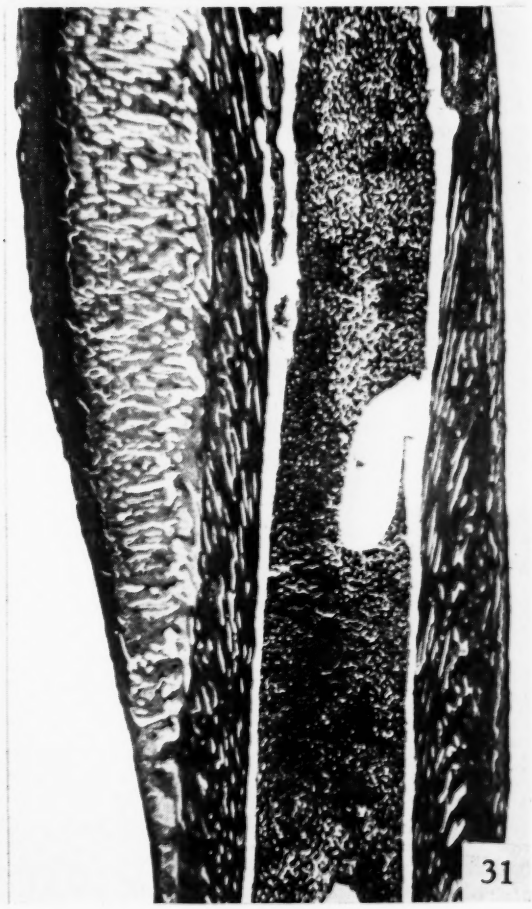


31

FIGS. 28, 29, 30, 31

Microscopically the osteopetrotic bones showed the same pathognomonic changes as described by Jungherr and Landauer (17) of which formation of strongly hyperchromatic new bone, marrow fibrosis, and partial or total disappearance of the marrow spaces were the most conspicuous (Figs. 34 and 35).

No definite sarcoma was observed. In only one case was it possibly present. Histological evidence from the cases of osteopetrosis indicates that the inner layer of the periosteum is the source (or one of the sources) of the new bone, and this opinion is strengthened by the study of the bone proliferation observed in association with the periosteal sarcomas (compare Figs. 30 and 32 with Figs. 34 and 35). Whether a previous sarcomatous lesion was indispensable for the development of osteopetrosis or whether this condition can develop from the inner layer of the periosteum as a reaction to the virus entirely independent of the neoplastic reaction of the outer layer is not known as yet. A decisive factor in favor of the latter alternative would be of course the presence in chicks of early bone proliferation in the absence of neoplasia. Although we did observe histological independence of both lesions in the same bone, proliferation in those animals was always associated with sarcoma growth. The question is then raised whether the cases of osteopetrosis represent these lesions at an advanced stage of their evolution. On the other hand that osteopetrosis can develop in the absence of demonstrable sarcomas is shown by the work of Jungherr and Landauer (17) and by our own experiments to be reported later.

The Nature of the Immediate Lesions Induced in Chicks by Duck Viruses

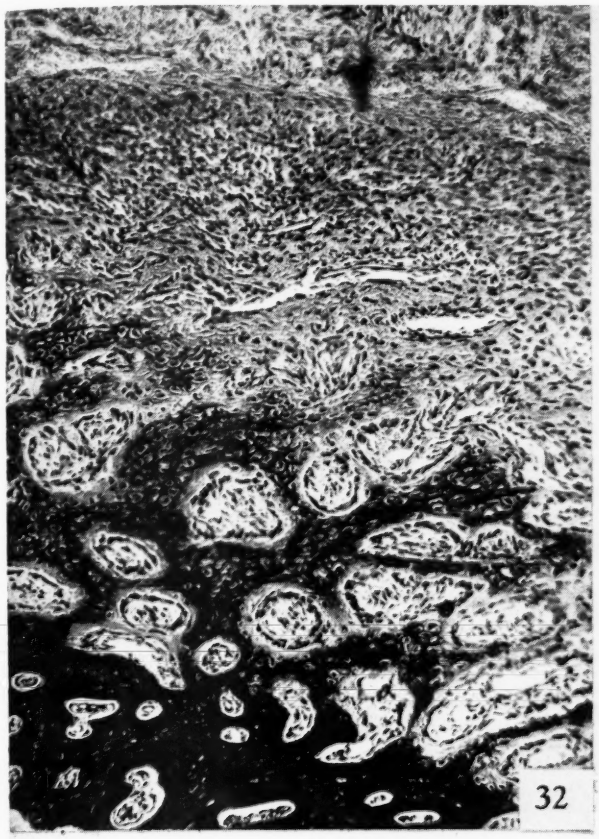
It will be remembered that in the chicken virus-duck sequence, the immediate tumors were solitary sarcomas looking much as the original Rous tumor does, and that the virus extracted from them was still a chicken virus because it induced tumors in chickens but not in ducks. Much the same thing occurred in comparable tumors in the duck virus-chick sequence. The analogies fundamentally present between the disease in chicks and that in ducks will be first considered.

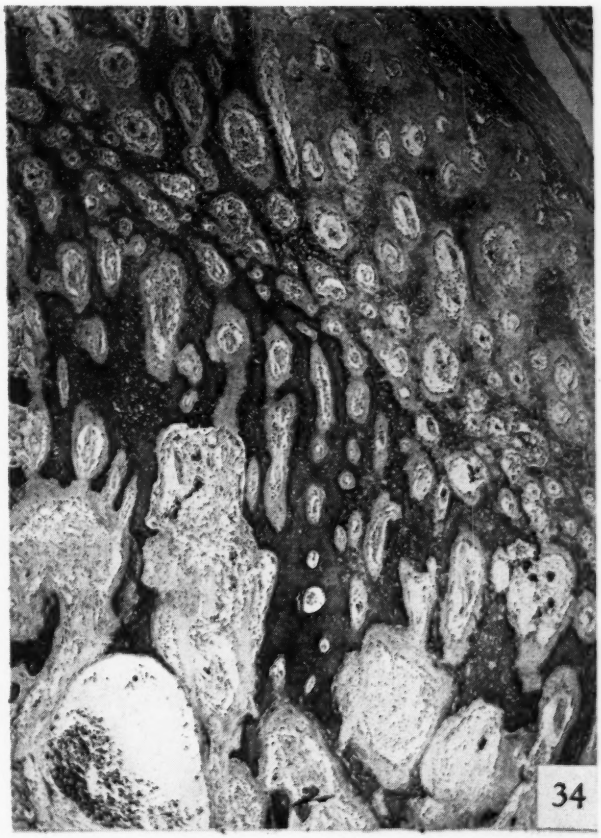
A very important trait common to strains HC and HV; namely, the development of periosteal and endosteal sarcoma, was faithfully reproduced in the infection of chicks, but the incidence of tumors induced by each strain in ducks and chicks was reversed. Strain HV induced 14 per cent of bone tumors in ducks and 45 per cent in chicks, whereas strain HC, from the 8th to the last passage, induced 41 per cent of bone tumors in ducks, 20 per cent in chicks. Moreover, there was in chicks a marked formation of new bone not observed in ducks. Also, in chicks injected with the strain HV the tumors always involved many bones whereas in those injected with strain HC the bone involvement was more moderate. A second important trait common to both strains of duck tumors; namely, the involvement of the digestive tract, was also reproduced but only to a limited extent in the infection of chicks, for only about 10 per cent of them injected either with the virus from ducks or with material from chick tumors of the subsequent passages developed lesions in the intestinal tract, mostly in the proventriculus and gizzard. In strain HC these lesions were often purely hemorrhagic. A third important trait common to both duck strains, the involvement of the skin, was practically lost in the infection of the chick, these lesions being found in less than 1 per cent of the injected chicks. On the other hand, the incidence of a pronounced hemorrhage-inducing power in strain HC, in contrast to its absence in strain HV, was directly reflected in the disease of chicks; the former strain induced 61 per cent of hemorrhagic lesions in ducks and 32 per cent in chicks, and the latter strain induced 10 per cent of such lesions in ducks and 0 per cent in chicks. The latter point is also brought up in Figs. 20 and 21. These observations emphasize the relative importance of factors either from the host or from the virus in determining the clinical manifestations of the neoplastic disease.

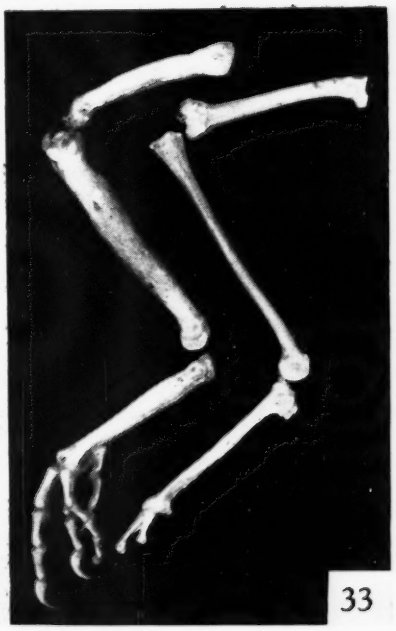
The transmissibility to both chickens and ducks of the immediate lesions shown by chicks injected with duck viruses was next taken up. Hemorrhagic lesions could not be transmitted in any instance, even when newly hatched animals were used. The subject will be developed elsewhere.

The following procedure was followed using the neoplastic lesions. Sublines of the tumors in chicks were started from the 4th, 6th, and 17th passages of strain HV and from the 19th passage of strain HC and carried out through both chicks and ducks. To secure good material for transmission the donors were killed and filtrates at 1:20 and cell suspensions at 1:5 were immediately obtained from tissue wholly free of necrosis. Details of the experiments and results obtained are represented in Figs. 36 to 39. In these figures we are now only concerned with the induction of immediate lesions—sarcomas and hemorrhage. Signs in the figures indicating leukoses or osteopetrosis will be considered later.

It is clear from Figs. 36 to 38 that none of the sublines from strain HV could be perpetuated beyond 3 generations of chicks while in subline 19 HC (Fig. 39) propagation was not accomplished beyond 6 generations. The age of the tumors used for transmission did not influence the results. The induction of lesions came to an end either gradually or abruptly. In the subline 19 HC (Fig. 39) the development of large primary tumors without metastases preceded the complete disappearance of the power to induce tumors. As shown in subline 6 HV (Fig. 37) ducklings injected with materials from the first chick passage developed a typical generalized disease which could be transmitted to other ducks for a presumably indefinite number of generations and the same was probably true after 2 chick passages. However a 3rd passage, attempted with a variety of materials from one of these







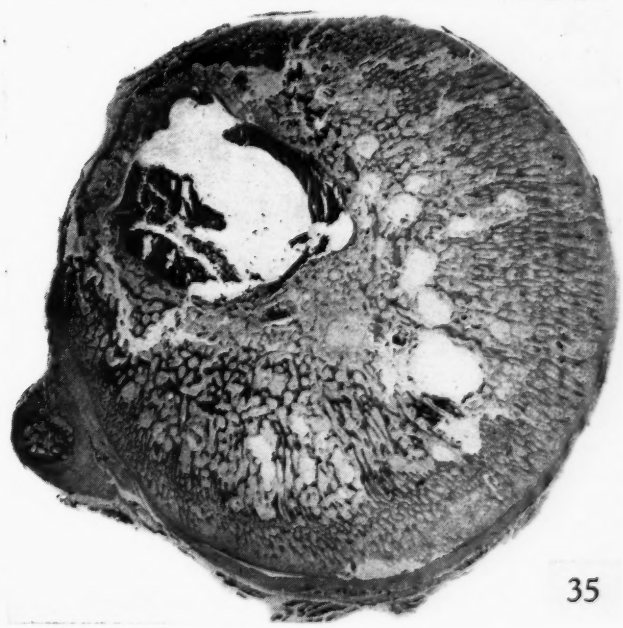


FIG. 32.—Section from another bone of same chick (see Fig. 28) showing sarcoma, at top, external to the intact periosteum, and marked proliferation of the inner layers of the periosteum with formation of new bony trabeculae. Mag. × 125.

FIG. 33.—Osteopetrosis of the leg bones of a chicken injected intravenously at the age of 1 day with virus from a tumor derived from the 4th passage of strain HV and killed 126 days later. The opposite leg was equally involved. Compare with bones of a normal chicken of the same age, shown on the right.

FIGS. 34 AND 35.—Longitudinal and transverse sections of the tibia of chicken with osteopetrosis. Mag. × 40 and 4.5.

ducks into chicks and other ducks, gave practically negative results in both species. Whether this was an accident or an indication of a modification of the infectivity of the strain even for ducks remains to be determined.

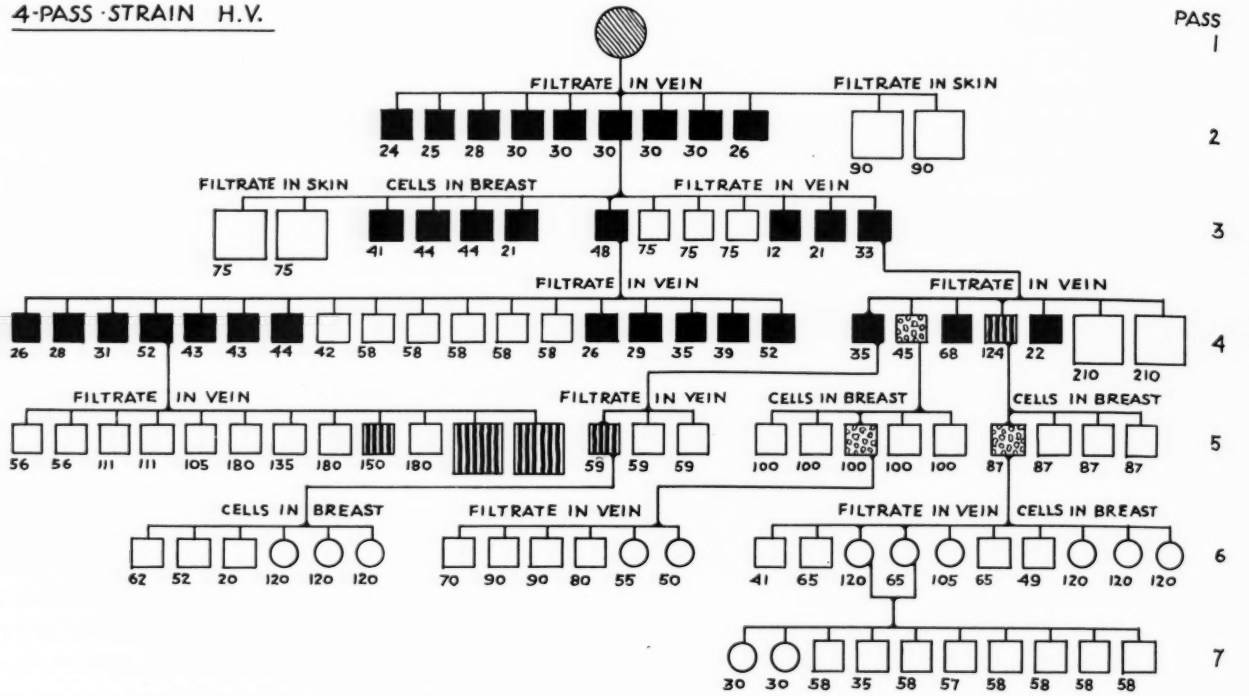


FIG. 36

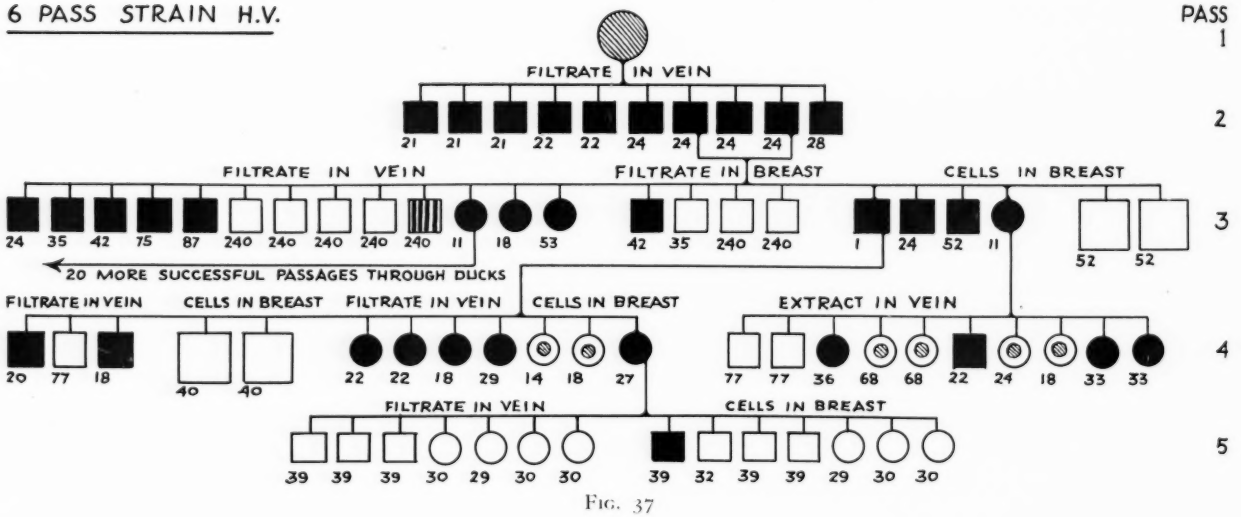


FIG. 37

Therefore, it is obvious that the viruses present in the immediate chick tumors were still duck viruses even after several generations through the heterologous host.

THE NATURE OF THE LATE LESIONS INDUCED IN CHICKENS BY DUCK VIRUSES

In the chicken virus-duck sequence these lesions were the expression of the transformation of the chicken strain into a duck strain of tumors, and the same seems to be true but in reversed order in the present case. In Figs. 20 and 21 it is shown that 4 cases of osteopetrosis and one of lymphomatosis appeared as a late effect of the infection of chicks with the original duck virus; in Figs. 36 and 37 it is shown that 3 cases of leukoses and 6 of osteopetrosis appeared as a late effect of the virus of the sublines which, as already stated, is a duck virus. The myeloid-like tumor is here considered as a late lesion. In the chicken virus-duck sequence the late sarcomatous lesions were easily transmissible through ducks, but the lymphoblastomatous lesions were transmitted with difficulty or not at all. The latter was also true in the opposite sequence

because a cell suspension at 1:5 of one of the leukotic tumors (the myeloid tumor of the ovary) induced a lymphoblastoma of the ovary in only one out of 5 chickens, and grafts (about 0.1 cc. of diseased bone and marrow) of one advanced case of osteopetrosis induced the same lesion in only one out of 6 chickens; a second passage of these lesions using chicks and also ducklings failed. However, in view of the results of Jungherr and Landauer (17) it is probable that if a

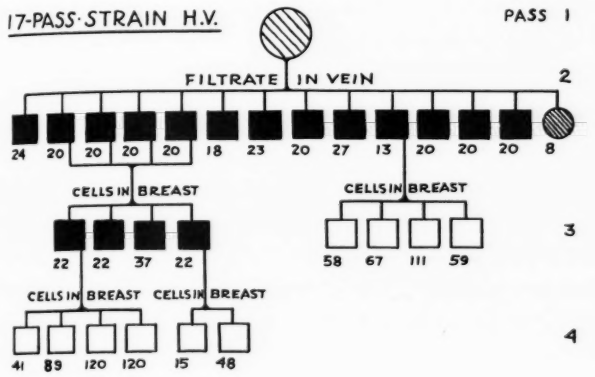


FIG. 38

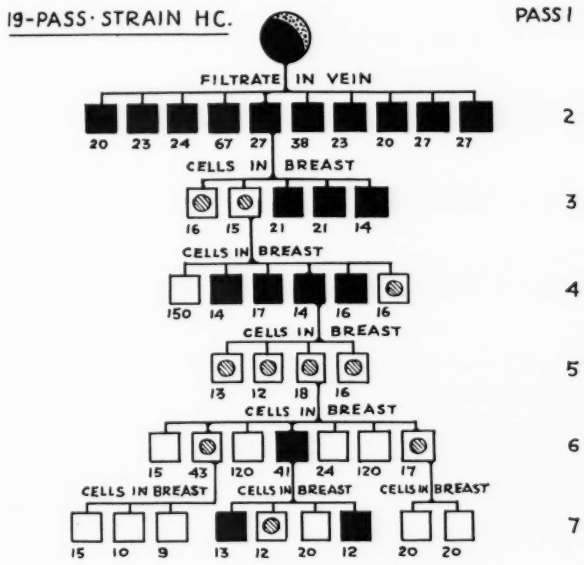


FIG. 39

larger number of chicks had been injected with materials from florid cases and if the injected animals had been kept long enough the passages could have been carried out further.

A difference of some importance in the development of the late lesions in chickens injected with duck virus as compared with those of the chicken virus-duck sequence is that adult animals may be susceptible. This is shown by the fact that one chicken injected with virus of the 28th passage of strain HV developed lymphomatosis (see Fig. 20), and 2 chickens 4 months old injected with virus from the chick subline 4 HV (Fig. 36) developed osteopetrosis. But time was nevertheless an indispensable factor for the infection of the adult because it took from 6 to 8 months for the lesions to develop.

Control Experiments

During the course of the present work, carried out during the last 2 years, we were well aware of the danger of attributing to a virus variation conditions which were the result of latent viruses in the ducks or chickens picked up during the passages. However, the regularity with which rare or heretofore undescribed lesions were obtained in most of our experiments on both ducks and chickens makes an objection of this sort untenable except in the case of lymphomatosis which is known to be a prevalent spontaneous disease in some flocks in Connecticut. But since this condition occurred in many of our experimental cases in association with osteopetrosis it is pertinent to point out that the latter condition never occurred spontaneously in the approximately 1000 Plymouth Rock chickens employed during the last $3\frac{1}{2}$ years in our work, and that spontaneous lymphomatosis itself was an extremely rare occurrence. Nor was leukosis or any other neoplastic disease found in many normal ducks studied when slaughtered in the farm supplying our stock or in about 2 dozen that died of acute conditions either on the farm or in our animal quarters. Moreover, at the same time that the experiments were carried out, we left a total of 20 chicks and as many ducklings without any treatment, and none of them showed neoplastic lesions when killed or when they died months later.

Nevertheless, control experiments were carried out; some of them were devised solely for this purpose, but some others were devised with the view of studying the possible transmissibility of "neoplastic" lesions which later proved not to be so. Eleven samples of different tissues from as many ducks and 6 from as many chickens were studied. Plain extracts or filtrates of these tissues were injected into a total of 32 ducklings, often within the first 24 hours after hatching, and into 22 chicks, and in 3 cases from 2 to 5 passages were carried out with extracts of tissues of the injected animals, using another 18 ducklings and 6 chicks. Some of the animals died of acute intercurrent non-neoplastic infections but most of them were killed from 3 weeks to 5 months after injection. In no case were tumors, leukosis, or osteopetrosis discovered at autopsies. One may well add that we had a similar experience when about 50 chicks were injected for the same purpose with a variety of tissues in connection with experiments undertaken before those reported here.

DISCUSSION

The essence of the findings is that the injection of ducklings with the virus of the Rous sarcoma of chickens induced two types of lesions: *immediate* tumors, which killed the host in a few weeks, and *late* tumors, which in most cases were not noticeable until several months later. The former infection involved no variation of the virus which remained a chicken virus, whereas the latter infection involved such variation, the virus passing to be a duck virus and inducing new types of tumors. Reversing the above sequence, the infection of chickens with duck variants of the Rous virus resulted, too, in the production of immediate and late tumors, the former involving no variation in the virus and the latter involving further variations with the production of still other types of tumors. Therefore, the infection that resulted in the production of immediate tumors was a *heterologous* infection, whereas that which resulted in the production of late lesions was a homologous infection. A summary of the results is given in Table V.

The heterologous infection by tumor viruses.—The main characteristic of this type of infection was its being strongly conditioned by several factors. Two of them; namely, immaturity of the host and high doses of the virus, were experimentally controllable; a third factor depended on inherent characteristics of the sample of virus used, and was uncontrollable within the limits of our experiments. These 3 factors were found indispensable in practically all cases. A fourth factor, the route of inoculation, was found important, but not indispensable. The conditioning was stronger in the infection of ducks by chicken viruses than in the opposite sequence. The fact that, within the limits of our experiments, only ducks within the first 24 hours after hatching were found susceptible, and that there was a detectable hourly increase in the resistance of the ducklings to the virus during this time was striking and can only be compared to the sudden acquisition of resistance by avian embryos in the last days of their development to heterologous viruses (1, 12) and tumors (20). The implications of the findings are obvious if one considers that the effects of this early infection cannot be manifested until later in life when the animal has reached maturity.

A second important characteristic of the heterologous infection was that the tumors retained in the heterologous host most of the features; *e.g.,* morphology, texture, and tissue affinities, that they showed in the homologous host. The *heterologous* tumors were generally localized in the chicken virus-duck infection, and generally widespread in the opposite sequence, but heterogenicity of the infection by no means implied mildness because practically all of our animals, ducks or chickens died of their tumors.

The third characteristic of the heterologous infection was that it could not be perpetuated, or only through a few generations, through the heterologous host, if cell-free extracts were used. If cells were used, then perpetuation of the heterologous duck tumors was possible, but these passages eventually led to variation of the virus; that is, to homologous infection. In chicks, the heterologous tumors died out after a few generations without ever reverting to the original Rous tumor. On the contrary, if virus or cells from the heterologous tumors were injected in the opposite host; that is, the host homologous to the tumor, then the original disease, capable of indefinite transmission, was restored.

Data concerning the length of the experimental heterologous infections, where large amounts of virus were injected, are indicated in Table V. One may well speculate on the possibility of spontaneous tumors occurring in nature following a comparable infection with smaller amounts of virus, and on the time a tumor can grow in a heterologous host without its virus undergoing variation. The problem then would be to find the host homologous to the tumor so that the etiological agent would be brought up.

TABLE V: HOMOLOGOUS AND HETEROLOGOUS INFECTION OF DUCKS AND CHICKENS BY TUMOR VIRUSES

Rous virus → ducklings	9-30 days →	Immediate lesions [No virus variation]	Lesions obtained: Hemorrhagic disease; Myxosarcomas; Spindle cell sarcomas
	40-215 days or longer →	Late lesions [Virus variation]	Lesions obtained: Spindle cell sarcomas (collagenous); Giant cell sarcoma of bone; Peri- and endosteal spindle cell sarcoma; Sarcoma-lymphoblastoma; Lymphoblastoma
Duck virus → chicks	9-100 days →		Lesions obtained: Hemorrhagic disease; Spindle cell sarcomas (collagenous); Peri- and endosteal spindle cell sarcoma
	45-215 days or longer →		Lesions obtained: Myeloblastoma; Lymphoblastoma; Osteopetrosis

The age resistance of ducks and chickens to cells from heterologous hosts is only slightly inferior to that to the virus, and as it happened with the latter, the conditioning by age of the infection of ducks by chicken tumor cells was more pronounced than in the inverse sequence. Allowance being made for these differences, it is clear that, whatever the defensive forces of the host are, they are equally directed against both tumor virus and tumor cells.

Variation of the tumor viruses and homologous infection.—Once the tumor viruses have implanted themselves in the heterologous host, *time* was found to be the only additional conditioning factor for the virus to vary and the infection to become homologous.[6] Rapidity of growth of the tumors was not indispensable because some of the late tumors, due to the variant virus, were several months old and yet they were quite small. It is obvious that homologous infection began when heterologous infection ended. But in the strains obtained by repeated passages of cells from the Rous tumor through ducks one could observe a gradual transition of the chicken type to the duck type, the new variant taking finally the upper hand. These tumors can well be considered as experimentally induced "mixed" tumors, and it is not inconceivable that some spontaneous tumors may contain a mixture of both homologous and heterologous viruses.

Is maturity of the host indispensable for the virus to vary and induce late lesions or are those the result of an evolution of the immediate lesions? Variation of the Rous virus into a duck virus has been repeatedly accomplished, by means of cell passages, in young ducks. But we do not know whether variation would have eventually occurred in all the tumors induced by the Rous virus in ducks had not the animals died of the toxic-like effect from the growth or whether the late tumors were induced by special and rare units of virus endowed with a potential ability to vary. Nor do we know for certain, although the second alternative seems far more probable, whether late lesions in chickens developed from unnoticeable immediate sarcomatous lesions or whether they developed from healthy tissue. Nevertheless, since in many cases a relatively long sojourn of the tumor in the heterologous host was indispensable for variation of the virus to occur, it is clear that such phenomenon is most likely to take place in maturity.

[6] When the preliminary note (6) on the subject was published only the immediate hemorrhagic lesions and the late sarcomas had been studied for transmissibility to adult chickens, and negative results were obtained in both cases. This led to the partly erroneous statement that apparently the change in the virus takes place as soon as the chicken virus has infected the duck cells. Nor was it then known that the infection of chicks by the duck variants may result in further virus variations and induction of late lesions.

The variant virus showed important changes which were best shown in the chicken virus-duck sequence resulting in late sarcomas. Besides the obvious morphological differences and the acquisition of new host affinities there was an acquisition of new tissue affinities. Thus, the original Rous virus shows a selective affinity for several viscera while bones, skin, and digestive tract are never affected, whereas most strains of duck viruses showed a selective affinity for the latter structures while still inducing tumors in viscera affected by the original virus. The entirely new texture of the duck sarcomas compared to that of the original Rous tumor reflects a profound alteration in the metabolic activity of the fibroblasts. To all these differences one should add others concerning the antigenic constitution of the chicken and duck viruses, but this will be taken up in another publication.

As for the transmissibility of the late lesions through the new host, now the homologous host, the following may be said. There was a variable capacity in the tumors to permit transmission depending on some inherent quality of the virus or cells. Sarcomas were most easily transmitted, and some of the strains are now at their 40th generation; next was the intermediate sarcoma-lymphoblastoma; and lastly, the lymphoblastomas, the myeloid tumor, and the osteopetrosis. Since only late lesions of the latter type were obtained in the duck virus-chicken sequence one may erroneously infer that the poor transplantability was somehow connected with the way these tumors originated, but lymphoblastoma in the chicken virus-duck sequence has also proved to be not transmissible so far. However, following the findings of Pappenheimer and Seidlin (23) it is known that at least some strains of lymphomatosis are transmissible, although far less readily than most sarcomas, provided certain requirements are fulfilled (8, 16, 17, 19). Although inherent characteristics (alluded to above) of the growths may be at play in our case, it is probable that better results would have been obtained by improving the methods of transmission. This is now done.

In the chicken virus-duck sequence, as the virus varied and the infection became homologous, the factors conditioning the heterologous infection became of much less importance. Ducks of practically all ages were infected with the passage virus although young individuals were more susceptible; the amounts of virus required were much smaller, and injections into the breast were as effective as intravenous injections. In the duck virus-chicken sequence our data are too scant to allow generalization. If we judge by the results obtained by ourselves and other authors in the transmission of lymphomatosis and osteopetrosis, it may well be that some of the conditioning factors are

still at play, and among them the youth of the host seems to be the most important (15, 17, 19).

The phenomena described in this paper are to be correlated with variations occurring among animal and plant viruses, and emphasize the great instability of these agents of disease. They bear closely on variations described by Kidd and Rous (18) attending the infection of the domestic rabbit by the virus of the Shope papilloma, and on the lack of transmissibility of this tumor through domestic rabbits, as shown by Shope (28). They may well deserve consideration in the interpretation of those strains inducing both sarcomas and leukoses, and may harmonize opposite views concerning the unity or plurality of viruses inducing leukoses and tumors (9-11, 15-16, 19, 22, 29). For while it is true that tumors have been experimentally induced containing "mixed" viruses, it is no less true that one of them was the result of a previous variation of the other, and the combination of viruses that can be thus obtained seems to be limitless.

The findings extend views by previous workers on the lack of species-specificity of avian tumors contrary to what was admitted for such a long time. The ease with which, starting from the Rous virus, so many different strains of tumors have been obtained in ducks and chickens and also, if it may be here anticipated, in turkeys and guinea hens, weakens the objection often heard against the virus theory of cancer; namely, that one would be obliged to suppose that there is a different causative virus for every different type of tumor. In fact, in our experiments, there is a different virus in each tumor, and what is more, the great capacity for variation found in the viruses of these bird tumors suggests the possibility of obtaining regularly by experimental methods types of tumors never so far observed in nature. Probably this already has been done in the present investigation.

SUMMARY AND CONCLUSIONS

It was possible to infect ducks with extract of the Rous sarcoma provided that the animals were injected within the first 24 hours after hatching with large doses of virus, preferably by the intravenous route. Of the 109 ducklings thus injected, 22 per cent developed lesions. There was a detectable hourly increase in the resistance of the ducks to the virus, the greatest susceptibility being found at hatching. By and large the resistance of the ducks to tumor cells was parallel to the resistance to the virus, the period of susceptibility to the former being only a few days longer than that to the latter.

The lesions induced by tumor extracts have been divided into *immediate* and *late,* the former lesions also including the tumors induced by suspensions of tumor cells. Immediate lesions developed within the first 30 days following inoculation, and consisted of solitary, myxo-, or compact spindle cell sarcomas very much like the original Rous tumor. Late lesions consisted of 3 different sarcomas and 1 lymphoblastoma in various locations. These tumors were generally found several months after injection when the ducks bearing them, mature animals by that time, were killed.

Extracts from immediate tumors reproduced in chicks and chickens the disease usually induced in them by the Rous virus, but these extracts were wholly ineffective in ducks. In other words, the virus causing the immediate tumors was still a chicken virus and the infection it induced in ducks was a *heterologous* infection. On the contrary, extracts from the late sarcomatous tumors had entirely lost their power to induce sarcomas in adult chickens, whereas they brought about a generalized disease in other ducks of which the most prominent feature was the induction of periosteal sarcomas with little or no formation of new bone. Two strains of duck tumors have been thus secured. In these cases the virus had varied, and the resultant infection was an homologous infection of the duck.

Variation of the Rous virus has also been obtained by repeated passages of tumor cells through young ducks starting with a cell suspension of the Rous tumor, and 4 more strains of duck tumors were thus obtained. In the course of these passages gradual transformation was observed from the chicken strain into a duck strain manifested by the progressive development of a generalized disease in the duck paralleling a progressive diminution of the disease in chickens.

Reversing the above sequence it was possible to infect 40 per cent of a total of 175 chicks a few days old with viruses from 2 of the duck variants, and this infection resulted, too, in the induction of either immediate or late lesions. The former developed within the first 100 days after injection and consisted of spindle cell sarcomas mostly arising from the periosteum—accompanied by a pronounced formation of new bone—and still other tumors much as in the disease of ducks. The latter consisted of leukoses and the condition known as osteopetrosis, these lesions not being noticed until several months after inoculation.

The immediate tumors could be transmitted by cells or extracts only through a few generations of chicks, the tumors eventually dying out, but if injected into ducks, then the original disease of this host was entirely reproduced. The virus causing these tumors in chicks was still a duck virus, and here again the infection of the animal was a heterologous infection. If cells from the duck tumors were injected into adult chickens, tumors developed on some occasions which were always followed by regression, and healthy tissue se-

cured by biopsies from these tumors failed to grow in other chickens.

The late lesions could be transmitted only to another generation of chicks, and also to a few adult chickens, the failure of indefinite propagation being attributable to causes which are analyzed. Injections of leukotic lesions induced leukoses but also osteopetrosis, and the reverse was also true.

Practically all the tumors in ducks and chickens resulting from either heterologous or homologous infection grew steadily until they killed their hosts. Death also resulted from the hemorrhagic lesions which quite frequently developed together with the neoplastic lesions, but this subject was only studied in passing.

The great variability, from 0 per cent to 100 per cent, in the infectivity of chicken and duck viruses for heterologous hosts even when all the requirements for successful infection were fulfilled, the great variety of tumors obtained, and still other facts described, emphasize the instability and the great capacity for variation of the viruses inducing the tumors here studied; and the implications of the findings in the tumor problem are discussed.

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Conditions Required to Produce a Prolonged Hypothermia in the Mouse*

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Four papers (1-4) have appeared describing the influence of lowered body temperature, or the influence of the attempt to produce lowered body temperature, upon the growth of sarcoma 180. It is the purpose of this note to clarify the interpretation of the data recorded and to submit additional data dealing with hypothermia in the mouse.

The conditions necessary to produce a state of continuous hypothermia in the mouse were reported from this laboratory (1-3) as follows:

Hypothermia is not produced at environmental temperatures of 10 to 15° C. below room temperature maintained for 10- to 15-day periods. Hypothermia is produced by subjecting mice to sudden low environmental temperatures, —2 to —5° C. Shivering occurs down to a skin temperature of about 20° C. In the range of skin temperature (inguinal) 15 to 20° C., the animals lose consciousness, respiration is considerably lessened and erratic, and the heart beat is reduced to about one-half normal. About 2 hours are required to arrive at this stage. When the hypothermic state is reached the mice may be maintained in it for 24 hours (body temperature below 20° C.) by placing them in an environmental temperature of approximately 15° C. The variation in temperature that might be expected in different parts of the body is not observed (Example: inguinal 19.4° C., peritoneal 17.9° C., thoracic 18.1° C., heart 19.2° C.). A considerable number of animals do not survive the treatment, and the success of the venture depends on constant vigilance during the hypothermic state as well as during the cooling period.

The conditions used by Goldfeder (4) follow: A temperature (environmental) of about 5 to 7° C. for a period of 8 to 24—and in a few instances 48—hours was found to be the limit of endurance. The rectal temperatures of the mice were determined before exposure to cold and immediately after their removal from the refrigerator. When a mouse was shivering intensely or was almost motionless, apparently near death, it was removed from the refrigerator and the refrigeration was resumed when the behavior appeared normal. The rectal temperature of mice varied from 29 to 31° C. The mice consumed a certain amount of food.

Our experience would indicate that the temperatures of 29 to 31° C. reported by Goldfeder were not attained for sustained periods of time and probably only at the end of the refrigeration period. It is stated that the mice were removed when shivering intensely or when motionless; viz., Goldfeder's experiment terminated at the point where experiments in this laboratory began. In our experience, mice maintained at an environmental temperature of 5 to 7° C., as was done in the experiments of Goldfeder, are able to maintain body temperature only slightly below normal for considerable periods. This adjustment occurs at expense of endogenous calorie-supplying depots and by increase in caloric intake. We believe that Goldfeder's experiment is in reality a study of the influence of the effect of low environmental temperature in increasing metabolism. Such a study was reported from this laboratory (1) and the Goldfeder experiments on tumor growth should be interpreted in this light, rather than on the basis of a prolonged hypothermia. When such an interpretation is made the anomalies in respect to respiratory quotients disappear.

EXPERIMENTAL

The experimental conditions described by Goldfeder were followed. Temperature was measured by means of a specially constructed rectal thermocouple, for which we are indebted to Dr. A. E. Koehler. The only difference in procedure was that the mice were removed from the container at intervals (2 to 4 hours) to record rectal temperature. The rectal temperatures of 4 mice weighing 25 to 27 gm. at the beginning of the experiment were 37.8, 38.2, 35.5, and 36.7° C. respectively. In two of the mice, the lowest rectal temperatures recorded, during exposure to an environmental temperature of 5 to 7° C. for 24 hours, were 35.4 and 36.1° C. After 48 hours' exposure the temperature of one of these mice fell to 34.0° C. In two other mice the temperatures fell to 32.8° C. only after 36 hours' exposure, but rose to 35.4 and 35.1° C. in the ensuing 12-hour period of exposure. The mice consumed 9 gm. of calf meal (1 gm. = 3.3 calories) per mouse in 24 hours. This is just double the average food consumption at room temperature. In the experiments, reported from this laboratory, in which a continuous hypothermia was produced, no food was consumed as the mice were unconscious during the period of hypothermia.

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SUMMARY AND CONCLUSIONS

The rectal temperature of mice, which are maintained at an environmental temperature of 5 to 7° C. and which have access to an adequate supply of foodstuffs, does not fall immediately to temperatures below 33° C. but may be maintained in the lower limits of the normal range for 24 to 48 hours. This adjustment occurs at expense of endogenous calorie-supplying depots and by means of increase (about double) in caloric intake. The influence of these experimental conditions upon tumor growth should be interpreted on the basis of a profound increase in metabolism, without losing sight of the derangement of the metabolic and catabolic processes which obviously ensue. To ascertain the immediate effects of hypothermia on tumor growth the period of increased metabolism can be reduced to 2 hours or less by using an environmental temperature −2 to −5° C., entailing a loss of body heat which exceeds the capacity for adjustment.

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The Cholesterol Content of Normal and Enlarged Prostates

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The increase in the cholesterol content of malignant tumors as compared with that of normal surrounding tissues has been noted by Roffo (12), Jowett (10), and Vargas (17). These observations have been confirmed by Yasuda (19), Bierich, Detzel, and Lang (3), and Burgheim and Joel (6) who also found the cholesterol content of benign tumors to be considerably less than that of the malignant type of growth. Burgheim (5) and Chahovitch (8, 9) have shown that carcinomas are strongly positive to the Schultz histochemical reaction for cholesterol (14-16). Cathie (7), however, was unable to find any correlation between malignancy and a Schultz positive reaction.

During the course of an investigation into the histology of enlarging prostates it was found that in most cases the Schultz reaction is more positive in the hyperplastic regions than elsewhere, the green color being most intense in the glandular cells themselves and much more marked than in normal prostates. It was therefore of interest to make quantitative observations on the cholesterol content of normal and enlarged prostates.

MATERIAL AND METHODS

The prostates were all obtained at post-mortem from the Radcliffe Infirmary, Oxford, and were fixed for about 5 days in formol saline. In some instances large adenomatous nodules were enucleated completely in order to compare their cholesterol content with that of the remainder of the prostate. On account of the uneven distribution of cholesterol in the prostates, as evidenced by the Schultz reaction, it was necessary to extract the whole prostate in every case (one frozen section was taken for histological study from a few of the specimens). The tissues were cut into small pieces not exceeding a few millimeters in any dimension and dried to constant weight at 100° C. They were then extracted with boiling chloroform under reflux until no further significant amounts of cholesterol could be removed. With prostates of average size; *i.e.*, not exceeding about 7 gm. dry weight, two separate extractions of 24 hours with about 40 cc. of chloroform for each proved sufficient; but larger amounts of tissue required correspondingly prolonged extraction. The combined extracts were then made up to known volume.

The cholesterol content of the extracts was determined colorimetrically by the Liebermann-Burchard reaction using a Pulfrich photometer. For color development, 10 cc. of the solution were taken and 3.8 cc. of acetic anhydride and 0.2 cc. of concentrated sulfuric acid were added. The mixture, in a stoppered flask, was then incubated at 37° C. for exactly 10 minutes and the first reading taken at 14 minutes, a red filter (610 A.U.) being used (see Appendix). The cholesterol concentrations were read off from a calibration curve, the standard cholesterol sample used in its construction having been triply recrystallized from alcohol.

RESULTS

The cholesterol contents, expressed in mgm. per 100 gm. dry tissue, of normal prostates and of those showing hyperplasia are given in Tables I and II, respectively. The mean cholesterol content of enlarged prostates is seen to be higher than that of normal prostates, the difference being significant statistically ($n=27$, $t=3.78$, $P=<0.01$).

In Table III, the cholesterol content of the "adenomas" from 9 enlarged prostates is compared with that of the remainder of the prostate in each case. From the mean ratio of cholesterol content of adenoma/cholesterol content of remainder it will be seen that the concentration of cholesterol in the adenoma is about twice that in the remainder of the prostate (the difference is significant statistically). It can also be seen that the mean cholesterol content of the remainder is closely similar to that of normal prostates.

DISCUSSION

It is difficult to evaluate the significance of the increased cholesterol content of enlarged prostates. Attention was drawn to the occurrence in tumors of crystals of cholesterol in loose combination with lecithin by White (18) as long ago as 1909, and the suggestion was put forward that cholesterol might be associated in some way with the regulation of cell proliferation. Subsequently, several workers have

TABLE I: CHOLESTEROL CONTENT OF NORMAL PROSTATES

Number	Age	Dry weight, gm.	Total cholesterol, mgm.	Cholesterol, mgm. per 100 gm. dry tissue
2	37	4.660	33.0	710
3	20	3.464	38.0	1095
5	25	2.806	8.0	285
8	58	3.742	33.0	880
10	19	2.849	27.5	965
11	45	5.092	40.0	785
13	61	5.337	37.5	705
15	40	4.840	48.0	990
16	42	3.181	28.0	880
18	54	5.378	45.5	845
21	32	3.305	27.5	830
25	46	5.988	46.5	775
26	53	3.409	22.5	660
Mean (13 specimens)				800

TABLE II: CHOLESTEROL CONTENT OF ENLARGED PROSTATES

Number	Age	Dry weight, gm.	Total cholesterol, mgm.	Cholesterol, mgm. per 100 gm. dry tissue
1	69	24.470	330.0	1350
4	72	18.381	197.0	1070
6	61	3.647	56.0	1540
7	48	5.072	48.0	945
9	71	11.876	116.0	980
12	64	4.001	49.0	1225
17	58	7.433	95.0	1280
19	65	7.099	100.0	1410
20	76	6.222	54.5	880
24	67	3.360	33.0	985
27	..	8.552	61.5	720
28	85	11.491	114.0	995
29	61	4.302	34.9	810
30	76	5.788	71.0	1230
31	57	6.171	64.0	1040
32	74	6.302	74.5	1180
Mean (16 specimens)				1102.5

demonstrated such a correlation. Thus, Robertson and Burnett (11) found that when cholesterol in dilute alcoholic or sodium oleate solution was injected into tumors, both primary and metastatic growth were accelerated, the acceleration of growth of the primary tumor being most evident in the premetastatic stage. Roffo (12) found that a fixation of cholesterol occurred in neoplasms, while in malignant disease there was an increased activity on the part of organs producing cholesterol. By keeping animals on a cholesterol-free diet, he found it possible to inhibit or prevent tumor growth. The same author (13) claimed to have demonstrated the absorption of cholesterol by neoplastic tissues *in vivo,* on the basis of a reduction in the cholesterol content of the blood leaving a tumor as compared with that of the arterial blood supplying it. On the other hand, Bennett (2) found that the injection of cholesterol into rats did not markedly affect the cholesterol content of carcinomas. He also found that the cholesterol content increased with age and that the outside or actively growing part contained less cholesterol than the central, less actively growing part, and concluded that a high cholesterol content was associated not with active growth, but with degeneration. It may be remarked that Bloor, Okey, and Corner (4) reached a similar conclusion in regard to the cholesterol content of the corpus luteum. The tumorigenic activity of cholesterol has also been doubted by Baumann *et al.* (1).

Although uncertainty still exists, it seems that the balance of evidence is probably in favor of cholesterol's playing at least some part in the growth of malignant tumors, and, from the evidence presented in this paper, in benign enlargement of the prostate. The possibility of hypercholesterolemia existing in patients with enlarged prostates was an interesting one, but in the few cases examined, the blood cholesterol was found to be within normal limits (about 180 mgm.

TABLE III: COMPARISON OF THE CHOLESTEROL CONTENT OF PROSTATIC ADENOMAS WITH THAT OF THE REMAINDER OF THE PROSTATE

	Adenoma			Remainder			
Number	Dry weight, gm.	Total cholesterol, mgm.	Cholesterol, mgm./100 gm. dry tissue (A)	Dry weight, gm.	Total cholesterol, mgm.	Cholesterol mgm./100 gm. dry tissue (B)	Ratio A/B
17	2.533	52.5	2070	4.900	42.5	870	2.38
19	2.318	48.5	2100	4.781	51.5	1075	1.95
20	0.730	12.0	1650	5.490	42.5	775	2.13
27	0.885	11.0	1240	7.664	50.5	660	1.88
28	5.374	82.0	1530	6.112	32.0	525	2.91
29	0.209	2.7	1290	4.093	32.0	785	1.64
30	1.644	29.5	1800	4.144	41.5	1000	1.80
31	1.178	19.5	1655	4.993	44.5	890	1.86
32	2.142	36.0	1680	4.160	38.5	920	1.83
Mean			1670			833	2.04

per 100 cc. whole blood). Further work is therefore awaited before the part played by cholesterol in prostatic enlargement can be appraised.

SUMMARY AND CONCLUSIONS

1. The Schultz reaction for cholesterol suggests that the adenomatous zones of enlarged prostates contain a high concentration of cholesterol, and that the cholesterol concentration of enlarged prostates is higher than that of normal prostates. These observations were confirmed by quantitative determinations.

2. The "adenomas" of enlarged prostates contain, on the average, twice as high a concentration of cholesterol as the remainder of the prostate.

when incubation was for 10 minutes and 5 minutes (Table IV included in Fig. 1).

A series of curves for color development was obtained by incubating successive samples for 2, 4, 6, 8, 10, 15, and 20 minutes and then following the color change in the photometer (room temperature 19° C.) (Fig. 1). The curves show the necessity for employing strictly standard conditions, not only in the period of incubation, but also in the times of the photometer readings, in order to obtain accurate results.

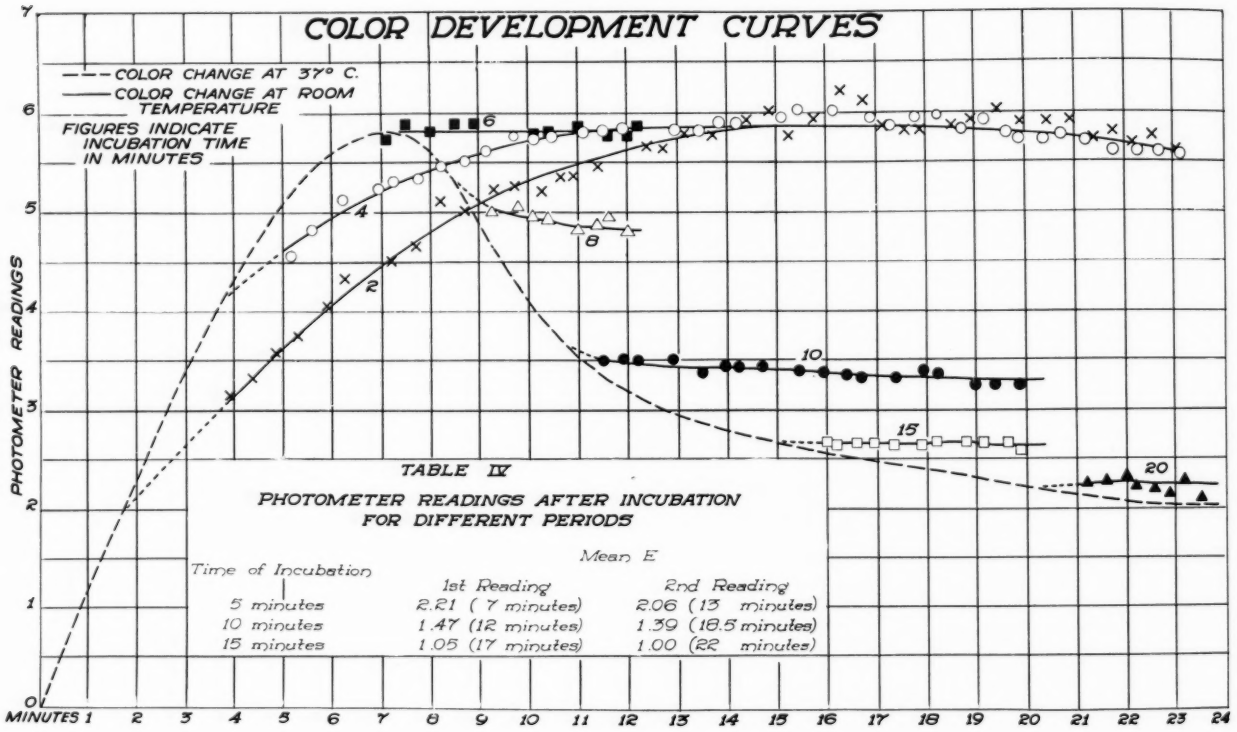

FIG. 1 and TABLE IV.—Color development curves in the Liebermann-Burchard reaction for quantitative determinations of cholesterol. The broken line represents the color change at 37° C.; the full lines represent the color change at room temperature (19° C.) after incubation at 37° C. for the following times: 2 min. = x; 4 min. = hollow circle; 6 min. = solid black square; 8 min. = hollow triangle; 10 min. = solid black circle; 15 min. = hollow square; and 20 min. = solid black triangle.

APPENDIX

Note on color development for photometry with the Liebermann-Burchard reaction.—The usual instruction for color development in the quantitative Liebermann-Burchard reaction; *e.g.*, as in Zeiss and Krebs (20), is to incubate for 15 minutes and then to take the highest reading. It was found that when this procedure was carried out the first reading was always the highest, and the same proved to be the case

I am indebted to Dr. A. Robb-Smith for providing the prostates; to Mr. J. R. O'Brien for affording me the facilities of the Biochemistry Department of the Radcliffe Infirmary, Oxford; and to my supervisor, Dr. S. Zuckerman, for valuable criticism. —AUTHOR.

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Correction

See: Jones, R. Norman. The Spectrographic Analysis of Carcinogenic Hydrocarbons and Metabolites. II. Determination of 1,2,5,6-Dibenzanthracene and 4′,8′-Dihydroxy-1,2,5,6-dibenzanthracene in Rat Excreta. Cancer Research, **2**:245-251. 1942.

P. 245, 5th line from bottom of first column.
For 4′,8′-dihydroxy-1,2,5,6-dibenzanthracene, read 4,8-dihydroxy-1,2,5,6-dibenzanthracene.

Abstracts

Reports of Experimental Research

Carcinogenic Compounds

CHARLES, D. R., and E. M. LUCE-CLAUSEN. [Univ. of Rochester, Rochester, N. Y.] THE KINETICS OF PAPILLOMA FORMATION IN BENZPYRENE-TREATED MICE. Cancer Research, 2:261-263. 1942.

The accumulation of papillomas in mice painted repeatedly with benzpyrene proceeds in approximate proportion to the square of the number of paintings, after an initial "lag period." Such a time course is shown to be consistent with the hypotheses that: 1. each painting causes some gene which is involved in normal differentiation of new skin cells to mutate to an inactive form in one out of every 18,000± stratum germinativum cells of the painted area; 2. any cell in which two such mutations have occurred thereafter gives rise to abnormal tissue which becomes recognizable as a papilloma after an interval which depends upon the growth rate of that tissue.—Authors' abstract.

du VIGNEAUD, V., J. M. SPANGLER, D. BURK, C. J. KENSLER, K. SUGIURA, and C. P. RHOADS. [Dept. of Biochemistry, Cornell Univ. Med. Coll., New York, N. Y. National Cancer Inst., Bethesda, Md., and Memorial Hosp., New York, N. Y.] THE PROCARCINOGENIC EFFECT OF BIOTIN IN BUTTER YELLOW TUMOR FORMATION. Science, 95:174-176. 1942.

To a diet highly protective against butter yellow tumors sufficient egg white was added to produce a borderline biotin deficiency. One group of rats was fed this diet alone, and another group received in addition 2.0 γ of crystalline biotin daily for 44 days, and 4.0 γ for the next 71 days. No tumors were found in the low biotin group at 6 months, whereas hepatomas were found in 3 of 5 rats receiving the crystalline biotin.

In two other experiments rats receiving crystalline biotin and two biotin concentrates of different strength also yielded more hepatomas than the controls. In the combined data of the three experiments only 1 out of 28 control rats had a liver tumor whereas 22 rats of a total of 50 receiving biotin developed such tumors.—M. B.

JONES, R. N. [The Converse Memorial Lab., Harvard Univ., Cambridge, Mass.] THE SPECTROGRAPHIC ANALYSIS OF CARCINOGENIC HYDROCARBONS AND METABOLITES. I. INTRODUCTION. Cancer Research, 2:237-244. 1942.

The principles of ultraviolet absorption spectrophotometry are discussed with reference to the application of spectrographic methods to the quantitative analysis of carcinogenic hydrocarbons and their metabolic products in tissue and excreta extracts. The data available concerning the absorption spectra of carcinogenic hydrocarbons and related substances are reviewed, and the factors limiting the accuracy and sensitivity of the method are considered. In tissue extracts, the presence of background absorption due to other absorbing constituents is a major factor in limiting the value of spectrographic analysis of such extracts and a rapid method of estimating approximately the degree to which such background interference is present in any given case, is presented.—Author's abstract.

JONES, R. N. [The Converse Memorial Lab., Harvard Univ., Cambridge, Mass.] THE SPECTROGRAPHIC ANALYSIS OF CARCINOGENIC HYDROCARBONS AND METABOLITES. II. DETERMINATION OF 1,2,5,6-DIBENZANTHRACENE AND 4′,8′-DIHYDROXY-1,2,5,6-DIBENZANTHRACENE IN RAT EXCRETA. Cancer Research, 2:245-251. 1942.

Spectrographic analyses of the feces and urine of rats which have received subcutaneous injections of 1,2,5,6-dibenzanthracene in olive oil indicate that of 4.5 gm. of hydrocarbon injected into 32 rats over a period of several weeks, only 231 mgm. could be detected in the excreta in an unchanged form and 43 mgm. as 4′,8′-dihydroxy-1,2,5,6-dibenzanthracene. Of this excreted material the greater part was found in the feces. The shape of the absorption curves suggests that the quantitative data on the hydrocarbon in the feces are more accurate than the other data, which are likely to err on the high side.—Author's abstract.

JONES, R. N. [The Converse Memorial Lab., Harvard Univ., Cambridge, Mass.] THE SPECTROGRAPHIC ANALYSIS OF CARCINOGENIC HYDROCARBONS AND METABOLITES. III. DISTRIBUTION OF 1,2,5,6-DIBENZANTHRACENE IN RATS FOLLOWING SUBCUTANEOUS INJECTION IN OLIVE OIL. Cancer Research, 2:252-255. 1942.

Spectrographic analysis of the organs of rats which had received subcutaneous injections of 1,2,5,6-dibenzanthracene in olive oil showed that considerable local storage of the hydrocarbon takes place in vesicles formed near the site of injection. Altogether some 36% of the amount of hydrocarbon injected could be accounted for in this manner. No spectrographic evidence of the presence of the phenolic metabolite, 4′,8′-dihydroxy-1,2,5,6-dibenzanthracene could be found in any of the tissues examined.

Qualitative experiments suggest that intraperitoneal injections of 1,2,5,6-dibenzanthracene in tricaprylin result in better absorption of the hydrocarbon than subcutaneous injections in olive oil.—Author's abstract.

Genetics

CASEY, A. E., L. PEARCE, and P. D. ROSAHN. [Rockefeller Inst. for Med. Research, Princeton, N. J., Sch. of Med., St. Louis Univ., St. Louis, Mo., and Sch. of Med., Louisiana State Univ., New Orleans, La.] THE ASSOCIATION OF BLOOD CELL FACTORS WITH THE TRANSPLANTABILITY OF THE BROWN-PEARCE TUMOR. Cancer Research, 2: 284-289. 1942.

In order to determine whether the transplantability, growth, and spread of the Brown-Pearce tumor might be influenced by factors reflected in the pretransplantation blood formulae, seventeen sets of experiments were carried out upon 195 young adult male rabbits, in apparent good health. Repeated determinations were made of the levels of the red blood cells, hemoglobin, platelets, total white

blood cells, neutrophiles, basophiles, eosinophiles, and monocytes. Seventy-nine animals were discarded for no other reason than that one or more average blood factor levels were abnormal, and 8 animals were discarded for other reasons. The final analysis was therefore carried out on 108 animals. After the blood examinations had been concluded, the animals were inoculated intratesticularly with the Brown-Pearce tumor. Transplantation was unsuccessful, by the criteria set up, in 18 animals.

No relationship could be demonstrated between the success or failure of transplantation and the pretransplantation levels of the blood platelets, neutrophiles, basophiles, eosinophiles, monocytes, or lymphocytes. A statistically significant relationship was, however, demonstrated between the success and failure of transplantation and the pretransplantation levels of the hemoglobin, the red blood cells, and the total white blood cells. The hemoglobin and red cell levels were interrelated and the relationship of the red blood cell level to the success of transplantation seemed secondary to the hemoglobin level. No relationship of the red blood cell level to the success of transplantation could be demonstrated when the hemoglobin and total white blood cell levels were held constant.

The resistance of the rabbit to transplantation of the Brown-Pearce tumor was found to be associated with optimal or modal pretransplantation levels of the hemoglobin and the total white blood cells. When the average level of either the hemoglobin or the total white cells (independent variables) was not modal the animals were susceptible. Besides the hemoglobin and total white cell levels, the presence of a third unknown or unidentified factor (or factors) was postulated.—Authors' summary.

FURTH, J., R. K. COLE, and M. C. BOON. [Cornell Univ. Med. Coll. and New York Hosp., New York, N. Y.] THE EFFECT OF MATERNAL INFLUENCE UPON SPONTANEOUS LEUKEMIA OF MICE. Cancer Research, 2:280-283. 1942.

Reciprocal crosses and reciprocal foster nursings were made between a high leukemia stock Ak and a low leukemia, high breast cancer stock C3H. The incidence of leukemia in the C3H/Ak hybrids was significantly lower (34% of 205 mice) than in the reciprocal F_1 generation (50% of 201 mice), and the difference was greater between the males (28% and 54% respectively). Foster nursing by low leukemia dams significantly lowered the incidence of leukemia in the high leukemia stock (from 58% to 27%), but the next generation behaved as nonfostered mice, having an incidence of leukemia of 59%. The reciprocal nursing failed to be productive of leukemia in the C3H mice. Leukemia tends to occur at a later age in F_1 hybrids than in the pure high leukemia stock mice.—Authors' abstract.

Radiation

AXELROD, D., P. C. AEBERSOLD, and J. H. LAWRENCE. [Crocker Radiation Lab. Univ. of California, Berkeley, Calif.] COMPARATIVE EFFECTS OF NEUTRONS AND X-RAYS ON THREE TUMORS IRRADIATED IN VITRO. Proc. Soc. Exper. Biol. & Med., 48:251-256. 1941.

The comparative effects of neutrons and x-rays were observed on a mammary carcinoma, a lymphoma, and a lymphosarcoma, all of the Strong A strain mouse. The subcutaneous tumors were removed aseptically and cut into fragments of about 10 mgm. each. These fragments were first wrapped in sterile filter paper moistened with sterile, buffered, physiological saline and then wrapped in cellophane; controls were similarly prepared. After irradiation, the tumor bits were inoculated subcutaneously by trocar bilaterally into the axillary region of A strain mice. These procedures were completed within 3 hours.

Neutrons were produced by bombardment of a beryllium target with deuterons accelerated to 16 million volts in the 60 inch cyclotron; the intensity was about 10 to 15 n per minute. The source of x-rays was a General Electric 220 kv. Maximar x-ray tube; the output was 61 r per minute.

The 3 control tumors grew 100% with but slight variation in growth rate. Both neutron and x-ray irradiation delayed the appearance of the tumors, the mammary carcinoma being the most affected. When the doses at the 50% survival point were compared, the x-ray/neutron ratios were 5.8 for lymphoma, 6.1 for mammary carcinoma, and 7.5 for lymphosarcoma. These ratios do not seem to indicate a tumor sensitivity to the radiations but show that per unit of ionization neutrons are more biologically destructive than x-rays.—M. B.

MORTON, J. J., E. M. LUCE-CLAUSEN, and E. B. MAHONEY. [Univ. of Rochester, Rochester, N. Y.] VISIBLE LIGHT AND SKIN TUMORS INDUCED WITH BENZPYRENE IN MICE. Cancer Research, 2:256-260. 1942.

Using the C57 black, Bar Harbor, strain of mice similar results were obtained to those of experiments already published where Swiss albinos were used. Mice kept for 12 hours daily in an environment of visible light, as compared with controls kept in complete darkness, showed delay in the appearance of skin carcinomas and a diminution in the total number which developed tumors. They were painted twice a week with 0.5% 3,4-benzpyrene in benzene (35 paintings). Eighty-nine per cent were found in the dark; 53% in the light. In addition, the C57 strain developed a dermatitis on exposure to light, characterized by intense irritation. The possibility of different rates of growth in skin cells in dark and in light, after painting, is discussed.—Authors' abstract.

Biochemistry and Nutrition—Chemotherapy

BELKIN, M. [Yale Univ. Sch. of Med., New Haven, Conn.] THE LACK OF INFLUENCE OF DI-(HYDROXYMETHYL) PEROXIDE ON THE INCIDENCE AND GROWTH OF TRANSPLANTED, INDUCED, AND SPONTANEOUS TUMORS. I. TRANSPLANTED TUMORS. Cancer Research, 2:264-268. 1942.

Eight hundred mice, 2 to 3 months old, of the ABC albino strain were inoculated with a suspension of tumor 15091a, a mammary carcinoma that grows in a high percentage of cases in this strain. The mice were divided into 8 groups of 100 mice each, each group alike with regard to age and sex.

The groups received the following treatment: group 1, injections of di-(hydroxymethyl) peroxide in concentration 1×10^{-3}; group 2, injections of peroxide 1×10^{-9}; group 3, injections of peroxide 1×10^{-18}; group 4, distilled water. Groups 5, 6, 7, and 8 served as untreated controls. The

animals in each of the first 4 groups received subcutaneously every 2 weeks 0.5 cc. of an aqueous solution of their respective peroxide concentrations or of distilled water. Injection was always made at a distance from the tumor.

The number of tumors, expressed as per cent of the effective total, which arose in each group are as follows: group 1, 94%; group 2, 95%; group 3, 93%; group 4, 92%; group 5, 94%; group 6, 95%; group 7, 94%; group 8, 89%. From the standpoint of number of tumors arising in each group, or incidence of these tumors with respect to time, no effect was evident of the administration of di-(hydroxymethyl) peroxide, either inhibitory or stimulating, in these transplanted tumors as has been reported by Maisin and his collaborators for tumors produced by benzpyrene painting. Also no difference was observed in the rate of growth or terminal weights of the transplanted tumors in any of the peroxide-treated groups as compared with the controls.—Author's abstract.

BELKIN, M. [Dept. of Pathology, Yale Univ. School of Medicine, New Haven, Conn.] THE LACK OF INFLUENCE OF DI-(HYDROXYMETHYL) PEROXIDE ON THE INCIDENCE AND GROWTH OF TRANSPLANTED, INDUCED, AND SPONTANEOUS TUMORS. II. TUMORS INDUCED BY CUTANEOUS PAINTING WITH BENZPYRENE. III. TUMORS INDUCED BY SUBCUTANEOUS INJECTION OF BENZPYRENE. Cancer Research, 2:269-275. 1942.

Four hundred mice of the Bagg albino strain, 2 to 3 months old, were painted in the interscapular area with 0.5% benzpyrene dissolved in benzene. Two drops were applied on alternate days, for a total of 50 drops after which benzpyrene treatment was discontinued. The mice were divided into 4 groups of 100 each, each group alike with regard to age and sex and given the following treatment: group 1, di-(hydroxymethyl) peroxide in concentration 1×10^{-4}; group 2, peroxide concentration 1×10^{-10}; group 3, peroxide concentration 1×10^{-19}; group 4, untreated controls.

A second series of 300 Bagg albino mice, 2 to 3 months old, was similarly divided into 6 groups of 50 mice each. They were painted with benzpyrene as described above, and each group received the following treatment: group 1, di-(hydroxymethyl) peroxide in concentration 1×10^{-3}; group 2, peroxide in concentration 1×10^{-9}; group 3, peroxide 1×10^{-18}; group 4, distilled water controls; groups 5 and 6, untreated controls.

Each treated group in the 2 series received subcutaneously every 2 weeks 0.5 cc. of an aqueous solution of their respective peroxide concentrations or of distilled water.

All animals were examined periodically for appearance of papillomas and a gross diagnosis was made for onset of malignancy. The per cents of malignant growths produced in each group, calculated from the effective total mice and based on microscopic diagnosis are as follows: series 1, group 1, 56%; group 2, 55%; group 3, 78%; group 4, 63%. Series 2, group 1, 90%; group 2, 61%; group 3, 61%; group 4, 63%; group 5, 66%; group 6, 55%.

The differences among the various groups are not considered significant, but of a magnitude compatible with normally variable yields of skin cancer in this strain under these experimental conditions. No prophylactic effect of peroxide administration was obtained in any group receiving this substance. Also, no significant difference was obtained in the incidence of the tumors with respect to time, or in the rate of growth of these tumors.

Histopathological examination of the malignant skin growths showed them to be epidermoid carcinoma. About 20% lung cancer was also encountered, as well as a few mammary cancers, fibrosarcomas, and squamous cell carcinoma of the stomach.

A preventive effect of di-(hydroxymethyl) peroxide on skin cancer produced by benzpyrene as reported by Maisin and his collaborators could not be confirmed, under the conditions of these experiments.

Eight hundred mice of the Bagg albino strain were each injected subcutaneously with 10 mgm. crystalline benzpyrene suspended in C. P. glycerine. They were then divided into 8 groups of 100 mice, each group receiving the following treatment: group 1, di-(hydroxymethyl) peroxide, concentration 1×10^{-3}; group 2, peroxide, concentration 1×10^{-9}; group 3, concentration 1×10^{-18}; group 4, distilled water controls; groups 5, 6, 7, and 8 served as untreated controls. The first 4 groups received every 2 weeks subcutaneously 0.5 cc. of an aqueous solution of their respective peroxide concentrations or of distilled water.

The number of tumors appearing in each group are as follows: group 1, 93%; group 2, 97%; group 3, 90%; group 4, 89%; group 5, 93%; group 6, 93%; group 7, 92%; group 8, 91%. From the standpoint of total numbers of tumors produced in each group, rate of tumor incidence with respect to time, and rate of tumor growth, no significant difference was observed in any group receiving peroxide as compared to the controls.

Histopathological examination showed the induced tumors to be fibrosarcomas, rhabdomyosarcomas, and epidermoid carcinomas. A few lung cancers were also obtained.—Author's abstract.

BELKIN, M. [Dept. of Pathology, Yale Univ. Sch. of Med., New Haven, Conn.] THE LACK OF INFLUENCE OF DI-(HYDROXYMETHYL) PEROXIDE ON THE INCIDENCE AND GROWTH OF TRANSPLANTED, INDUCED, AND SPONTANEOUS TUMORS. IV. SPONTANEOUS TUMORS IN THE DBA AND C3H STRAINS. Cancer Research, 2:276-279. 1942.

Two hundred and ninety-seven females of the dilute brown (Dba) strain were divided into 3 groups, each of which received the following treatment: group 1, 100 mice, di-(hydroxymethyl) peroxide in concentration 1×10^{-10}; group 2, 100 mice, peroxide in concentration of 1×10^{-19}; group 3, 97 mice, untreated controls. The first 2 groups received subcutaneously, every 2 weeks, 0.5 cc. of an aqueous solution of their respective peroxide concentrations. Also, each animal was permitted to have and nurse one litter to weaning age.

The number of spontaneous tumors (expressed as per cent of the effective total) arising in each group are as follows: group 1, 57%; group 2, 70%; group 3, 59%. Microscopically the tumors were preponderantly adenocarcinomas of the breast and lymphosarcomas, although a number of other types were also encountered.

In another experiment, 600 C3H females were divided into 6 groups of 100 each. Each group received the following treatment: group 1, di-(hydroxymethyl) peroxide

in concentration 1×10^{-3}; group 2, peroxide 1×10^{-9}; group 3, peroxide, 1×10^{-18}; group 4, distilled water; groups 5 and 6 served as untreated controls. The first 4 groups received subcutaneously every 2 weeks 0.5 cc. of their respective peroxide concentrations or of distilled water. Each animal was permitted to have 2 litters; the first was raised to weaning age, the second removed at birth.

The number of spontaneous tumors (expressed as per cent of the effective total) secured in each group are as follows: group 1, 42%; group 2, 46%; group 3, 45%; group 4, 52%; group 5, 39%; group 6, 55%. Histologically the tumors were preponderantly adenocarcinomas of the breast, although a few other types were also obtained.

In both experiments, from the standpoint of total number of spontaneous tumors obtained, rate of incidence of these tumors with respect to time, and rate of growth of these tumors, no significant difference was observed between those groups receiving di-(hydroxymethyl) peroxide and the control groups.—Author's abstract.

DUBOIS, K. P., and V. R. POTTER [Univ. of Wisconsin, Madison, Wis.] BIOCATALYSTS OF TUMOR TISSUE. I. CYTOCHROME C. Cancer Research, 2:290-293. 1942.

The authors determined the cytochrome *c* content of experimental tumors by means of their previously published technic. The following types of tumors were studied: Flexner-Jobling rat carcinoma, Jensen rat sarcoma, Walker rat carcinoma No. 256, Rous chicken sarcoma, butter yellow-induced rat liver tumor, spontaneous mouse mammary tumors, Yale mouse tumor No. 1, and transplants from tumors induced in mice by ultraviolet irradiation and by injection of 2,2′-azonaphthalene. The tumors contained from 10 to 20 μgm. of cytochrome *c* per gm. of fresh tissue regardless of etiology. Normal rat liver contains 90 to 100 μgm. of cytochrome *c* per gm.; that is, about 5 times as much as the butter yellow-induced rat liver tumor.—Authors' abstract.

COMPARATIVE ONCOLOGY

HUEPER, W. C., and G. J. MARTIN. [Warner Inst. for Therapeutic Research, New York, N. Y.] A TUMOR OF THE ADRENAL MEDULLA IN A CASTRATED MALE RAT. Cancer Research, 2:294-295. 1942.

The adrenal tumor described was found in one of 30 castrated male rats kept on a vitamin E-deficient diet and was apparently of medullary origin. The location of the tumor in the gland, the presence of nervous tissue elements in the neoplastic parenchyma, and also, to a certain extent, the occurrence of arteriolosclerotic lesions in various internal organs support this diagnosis. In view of the absence of chromaffinic matter in the cells of the neoplasm, the diagnosis of ganglioneuroma of the adrenal seems to be justified.

It is uncertain whether or not the development of this blastoma is causally related to the endocrine and vitamin disturbances experimentally produced in this rat. Investigations now under way may clarify this question, which is important, as Gardner concluded that the adrenal tumors observed in ovariectomized mice are of cortical derivation and originate from the zona glomerulosa; *i.e.*, remote from the androgenic zone.—Authors' summary.

Clinical and Pathological Reports

Diagnosis—General

GEMMELL, A. A. [Univ. of Liverpool, Liverpool, England] OBSERVATIONS ON THE COLLOIDAL VANADATE REACTION (BENDIEN REACTION) IN A SERIES OF CASES OF CARCINOMA OF THE CERVIX. Cancer Research, 2: 296-302. 1942.

The results of the colloidal vanadate reaction are presented for a series of 44 proven cases of cancer of the cervix. Particular attention was given to the correlation of the initial flocculation with the clinical conditions of the patients and the course of the cases. All patients except one were treated with radium. The test is not specific for the diagnosis of cancer but has prognostic value. Initial flocculation values which remain practically horizontal at the level 25 to 28 indicate good prognosis. Initial flocculation reactions showing a falling curve are of serious prognostic import. Initial flocculation values below 25 and readings of 23 or less are indicative of bad prognosis and will be found chiefly among those whose clinical progress is unsatisfactory.

The "fraction" was too variable under the conditions of this investigation to be of any value in prognosis.

Patients with a pretreatment initial flocculation of 25 or higher had a significantly higher survival rate. Under the conditions of this investigation the pretreatment fraction was not useful as a guide to diagnosis or prognosis.—From author's summary by S. B-J.

Ovary

BROWN, A. L., and M. SHOOR. [Mount Zion Hosp., San Francisco, Calif.] STRUMA OVARII. Am. J. Surg., 55: 173-174. 1942.

Report of ovarian tumor composed of thyroid tissue, without noticeable metabolic changes.—H. G. W.

JONES, H. W. JR., and G. E. S. JONES. [Johns Hopkins Hosp., Baltimore, Md.] MESONEPHROMA OF THE OVARY. Arch. Path., 33:18-27. 1942.

A study, based on 15 cases of which 9 are new, leads to the conclusion that mesonephroma is a pathologic entity but of uncertain histogenesis, and diagnosed only by histologic evidence. It is a highly malignant tumor, all but 3 of the 15 patients having died within a short time after operation. The authors' impression is that the tumors are epithelial in origin, but they are uncertain as to their mesonephric origin.—H. G. W.

LONG, C. H., J. ZISKIND, and A. H. STORCK. [Sch. of Med., Tulane Univ., New Orleans, La.] DYSGERMINOMA OCCURRING IN A PSEUDOHERMAPHRODITE. Surg., Gynec. & Obst., 73:811-818. 1941.

Among 68 solid ovarian tumors found at operation and autopsy, but one dysgerminoma was discovered. This occurred in both ovaries of a pseudohermaphrodite, and was removed without recurrence.—H. G. W.

Gastrointestinal Tract

GNASSI, A. M., and H. P. PRICE. [Jersey City Med. Center, Jersey City, N. J.] ADENOCARCINOMA OF THE RECTUM AND CHROMARGENTAFFINE TUMOR OF THE JEJUNUM. Am. J. Surg., 55:163-165. 1942.

Case report of dual cancer.—H. G. W.

HENNING, B. H., and L. H. GARLAND. [San Francisco, Calif.] LEIOMYOSARCOMA OF THE DUODENUM. Radiology, 37:353-356. 1941.

A case report of leiomyosarcoma involving the second and third portions of the duodenum of a 65-year-old woman. The patient died of myocardial failure 2 days after surgical removal. This is the 10th reported case of leiomyosarcoma of the duodenum.—C. E. D.

IVANISSEVICH, O., and R. C. FERRARI. [Buenos Aires, Argentina] CARCINOMA OF THE ESOPHAGUS. Surg., Gynec. & Obst., 74:47-52. 1942.

A case submitted to Torek's operation with recovery for 5 months.—H. G. W.

MAYO, C. W., and C. P. SCHLICKE. [Mayo Clinic, Rochester, Minn.] CARCINOMA OF THE COLON AND RECTUM. Surg., Gynec. & Obst., 74:83-91. 1942.

A study of metastases and recurrences in 334 patients subjected to post-mortem examination showed that the ability of the surgeon to detect metastases at the time of operation was good, for when death occurred shortly after resection no metastases were found in 82.4% of those in which none was found at the time of operation. No relation between the site of the tumor and the location of metastases in the liver could be found. Residual cancer was found in only 5.3% of the cases in which death occurred shortly after resection. In those cases in which the surgeon believed metastatic growth to be present in the liver the pathologist confirmed him in 91.7%. Independent growths are apparently responsible for many recurrences, for polyps were present in 34.1% of the cases in this series, in contrast to 16% of a control series of patients without cancer of the bowel, and in 14% malignant changes were present in the polyps. Multiple carcinomas were found in 8.4% of the cases. Among cases in which resection was carried out, additional independent cancers were found at necropsy in 4.1%.—H. G. W.

SVIEN, H. J., and A. B. RIVERS. [Mayo Clinic, Rochester, Minn.] LYMPHOSARCOMA CAUSING OBSTRUCTION AT THE DUODENOJEJUNAL ANGLE. Am. J. Digest. Dis., 9:45-47. 1942.

Case report.—H. G. W.

Leukemia

LEWIS, M. R., and G. B. MIDER. [Carnegie Inst. of Washington, Baltimore, Md., the Wistar Institute, Philadelphia, Pa., and Nat. Cancer Inst., Bethesda, Md.] THE IDENTIFICATION OF CELLS FROM INDUCED AND SPONTANEOUS LEUKOSES OF DILUTE BROWN MICE. J. Nat. Cancer Inst., 2:115-122. 1941.

Methylcholanthrene-induced leukoses in dilute brown mice have certain characteristics which are different from similar leukoses occurring spontaneously. These differences suggested that the induced leukoses and the spontaneous leukoses might have different origins. To determine whether this was so, motion pictures were taken of the cells as they grew in tissue culture. Differences in type of locomotion and activity were taken as characteristics of different myeloblastic and lymphoblastic types of cells.

In the spontaneous leukoses, rapid proliferation of the stroma occurred. The cells of one spontaneous leukosis moved along the stroma in a steady progressive gait and remained within the focus at all times. The nucleus progressed leaving a small caudal cytoplasmic projection. This type of action resembles that of lymphocytes. The cellular characteristics and locomotion of the second spontaneous leukosis resembled more nearly that of large lymphocytes and lymphoblasts found in normal lymph nodes and some lymphosarcomas.

The cellular locomotion and activity of the cells of the induced leukoses resembled that of normal bone marrow myeloblasts, leukemic blood myeloblasts, and cells of myeloblastic sarcoma. The cells were rounded in the resting stage but became elongate when the cell migrated. The elongate cells left the plane of focus and returned several times during observation. The motion was writhing and wriggling as has been shown to be characteristic of the myeloblastic series.—R. C. R.

Gastrointestinal Tract

SAYPOL, G. M., and J. W. HINTON. [New York Post Graduate Hosp., New York, N. Y.] STATISTICAL REVIEW OF CARCINOMA OF THE STOMACH. Am. J. Surg., 54:431-434. 1941.

One-fourth of 196 cases admitted to the New York Post Graduate Hospital were inoperable, with a mortality of 15.5%, as contrasted with cases admitted to Bellevue Hospital, of which two-thirds were inoperable with a mortality of 51.7%. The mortality of the operable cases was 35.1%, as contrasted with a mortality of 52% at Bellevue. The resectable cases comprised 25.5% of the total as compared with 5.4% of the total at Bellevue, showing the influence of social and economic status on the diagnosis of cancer of the stomach.—H. G. W.

STALKER, L. K., E. T. RULISON, and J. D. WHITE. [Rochester, N. Y.] THE ASSOCIATION OF DIVERTICULITIS AND CARCINOMA OF THE COLON. Am. J. Digest. Dis., 8:440-441. 1941.

A case is reported in which diverticulitis and carcinoma of the sigmoid were present together. The association is rare and there is no reason to believe that diverticulitis is a precursor of cancer.—H. G. W.

Pancreas

HALPERN, S. R., and G. J. FASHENA. [Children's Med. Center, Dallas, Texas] VON RECKLINGHAUSEN'S DISEASE WITH DIABETES MELLITUS. J. Clin. Endocrinol., 1:726-727. 1941.

The authors believe this is the sole report of a case in which these two conditions are co-existent.—J. B. H.

TRIMBLE, I. R., J. W. PARSONS, and C. P. SHERMAN. [Baltimore, Md.] A ONE-STAGE OPERATION FOR THE CURE OF CARCINOMA OF THE AMPULLA OF VATER AND OF THE HEAD OF THE PANCREAS. Surg., Gynec. & Obst., 73:711-722. 1941.

A successfully operated case is reported, in which deprivation of the pancreatic secretion by ligation of the pancreatic duct has for 11 months had no apparent effect upon the digestion.—H. G. W.